BULLETIN

OF

THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XXXIV—No. 391] BALTIMORE, SEPTEMBER, 1923 [Price, 50 Cents

CONTENTS

Entered as Second-Class Matter at the Baltimore, Maryland, Postoffice.
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918.

STUDIES OF LIVING HUMAN BLOOD-CELLS

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The technique of staining smears of blood by the various modifications of Romanovski's method, that is, by mixtures of azur, methylene blue and eosin dissolved in the alcohol which serves as a fixative, has reached so high a degree of perfection and the surveys of blood-cells by this method are so complete, that further progress must now depend on the use of some new method. Such a method is, I think, furnished by the differential staining of living blood-cells, and by this method a whole new range of hematological problems has been opened up.

The method of studying blood-cells with supravital dyes was applied by the writer [1] in 1921 to the study of developing blood-cells in the embryo chick, both within the vessels as seen in the area vasculosa, and in smears of the living cells. During the same year, Miss Miriam E. Simpson [2] made a study of the reactions of living blood-cells to the large group of dyes which Evans and Scott [3] had studied with reference to an analysis of the cells of the connective-tissues. She also used [4] the vital method to separate the group of monocytes, first, in human blood and second, in rabbits under experimental conditions.

The technique of studying living blood-cells is so simple, that it is readily applicable to the study of clinical cases. All that is needed is clean slides and covers, a few drops of a stain and a warm box for the microscope or warm stage incubator, so that the slides can be kept at body temperature. The details of the method are as follows: the coverslips are cleaned as for fixed smears. The slides are also cleaned by the usual technique, that is, they are kept for three or four days in concentrated sulphuric acid to which a few crystals of potassium bichromate have been added. From the acid they are placed in running water, for 2 to 3 hours, care being taken that the water gets between the slides so that all acid is removed. This is essential, since living cells are sensitive to an acid. They are then rinsed 2 to 3 times in distilled water in which they are left over-night, and

finally stored in 80% alcohol. Before using they are dried on a clean towel and given a high polish with jeweler's rouge applied on a piece of clean silk. For the suggestion of this method of polishing I am indebted to Dr. Edward Malone; it is a great advantage because it ensures an even film of the vital dye. To get an even film of the dye, flame the slide; be sure that it is free from dust and then flood it with a dilute solution of any dye dissolved in absolute alcohol, drain off the stain quickly and stand the slide upright until it is dry. The stains can be used over and over but must be filtered frequently to keep them free from dust. The film should be faint but spread evenly on the slide. The slides can then be stored until they are needed, care being taken to keep them free from dust. Slides with any grease will have blotches on the stain and should be discarded. In drying the slides, do not blow on them, for again the stain will be uneven; if the stain is uneven, some of the cells will get too heavy a dose and be killed or damaged.

For stains, I have used neutral red, a combination of neutral red and Janus green, brilliant cresyl blue and azur. For the most part I have used neutral red which is relatively non-toxic. The stock solution of neutral red is made by adding 100 mg. of the dye to 10 c.c. of absolute alcohol. I have used only the dye put up by Grübler before the war, marked *"Zu Injekt. i. vital Gew. n. Ehrlich."* For making the films on the slides, take 10 c.c. of absolute alcohol and add 0.4 c.c. of the stock solution. The strength of the solution can be estimated by the color after one has had a little experience; it should be a clear rose color. The proper strength of the dyes can be readily determined by testing them with normal living blood-cells; the slightest staining of the nucleus will indicate that the stain is too strong. For the neutral red Janus green mixture, take 2 c.c. of the dilute solution of neutral red and add 3 drops of a saturated solution of the Janus green in absolute alcohol. The staining qualities of the mixture last only a few days. This mixture is much more toxic than the neutral red. The eosinophiles are amœboid in it but the neutrophilic leucocytes are not. As Miss Simpson discovered, the Janus green has the very great advantage of being differential for the lymphocytic strain of cells, since all of the lymphocytes show a characteristic arrangement of mitochondria. Azur, which may be obtained by using about 10 to 12 drops of Wright's blood stain in 10 c.c. of absolute alcohol, is a convenient dye but much more toxic than neutral red. The brilliant cresyl blue originally prepared by Grübler is an excellent dye; but the American dye, which I have used, has been mixed with a red dye which is far too toxic for supravital use.

It will be readily recognized that the thickness of these films of dye must vary with the number of cells to be stained in the preparation. For example, in a film of bone-marrow where practically all of the cells take up the dye, the strength of the solution must be greater than for blood where only a small proportion of the cells take any stain. The same is true in studying smears of lymph glands and spleen. For smears of blood a solution of the dye, of such strength that it will stain all of the cells immediately, can be safely and readily used, while for smears of lymph glands, spleen and bone-marrow, better results are obtained with a dye that takes 10 to 15 minutes to react. In a film of embryonic blood, where every single red blood-cell also takes the vital dye, a stronger solution should be used. It should also be noted that the vital staining of the white blood-cells takes a much weaker dose of any dye than the staining of the reticular substance of the red blood-cells, so that some of the white cells may be damaged if all the red cells stain. This fact explains the difficulty of staining the reticular substance of the red cells in marrow, as contrasted with the method used for films of blood, since with the marrow a dilute stain may all be taken up by the white cells.

Films of blood are made on the stained slide exactly as for any fresh film of blood. They must be sealed immediately with a vaseline of high melting point, for which we use Salvoline. Formerly we used melted Salvoline, but have found it better to smear it, while cold, along the edge of the coverslip, lest the heat damage the cells. Vaseline mixed with paraffin can be used; the melting point must be above the temperature of the warm box, otherwise the vaseline will melt, mix with the immersion oil and make the film opaque. After the film has been sealed, it should be put into a warm box; it is not necessary to hurry in getting it into the box, for the cells will show active motility even after having been kept at room temperature for 15 to 20 minutes. This makes it possible to carry the slides from the ward to the laboratory. In case considerable time would have to elapse, an arrangement of carrying the slides on a hot-water bottle could be devised. It will be found that blood from which a considerable amount of fibrin is deposited on the slide tends to have the red cells clump. It is not necessary to have every single red blood-cell distinct as for stained smears, but very much clumping of the red cells makes a film in which the count of the lymphocytes will be too small. The leucocytes will wander from the clumps of red cells but the lymphocytes will not, and may therefore be missed in the count. Of course films with any rouleaux are too thick. As with fixed smears, the cells at the edges must not be counted because an excess of dye collects at the edges so that some of the cells may be killed or damaged.

Since the living cells are sensitive both to acid left on the slide and to the strength of the dye, each new box of slides and each new bottle of the dilute stain should be tested with normal blood to avoid any false inferences concerning abnormal blood. The cells should all stain characteristically without any staining of the nuclei.

We regard any coloring of the nucleus as an evidence of damage to the cell, regardless of the fact that the cytoplasm may continue to move a short time after the nucleus has begun to stain. The amount of motility and the duration of motility are certainly reduced.

The descriptions of the vitally stained cells which follow are limited to human blood; it has been found in the progress of experimental work on animals, which is now in progress, that there are greater variations between the blood-cells of different species than had been established by other methods. The observations on comparative studies will be brought out later. In normal human blood, every type of cell stains so characteristically with a vital dye, that there is rarely a cell that cannot be distinguished with certainty. In abnormal blood, on the other hand, when a given group of cells has been stimulated or damaged, its reactions to the vital dyes may be markedly changed; or when unusual cells have been called into the blood-stream, it may be difficult or even impossible to distinguish them. The difficulties are, I think, not greater than with fixed smears, at any rate they are not always identical with the difficulties in smears; in consequence there are great advantages in a combination of the two methods.

The very marked gain in the use of the supravital method for the study of the white cells is the possibility of distinguishing cells which have been markedly stimulated to activity from cells which are degenerating. The second great advantage is the ability to distinguish the old and dying cells so as to complete the life cycle of each of the three types of white cells.

In making these studies I am very deeply indebted to the physicians of the Johns Hopkins Hospital for the opportunity to study abnormal blood-cells. Through the courtesy of Dr. W. P. Longcope, Dr. H. L. Amoss and Dr. J. G. Huck I have been able to follow cases on the wards and Dr. W. G. MacCallum and Dr. A. R. Rich have allowed me to study material from autopsies in which individual cells were still alive. Without these advantages this work could not have been done.

TERMINOLOGY

There has been considerable discussion regarding the use of the terms, *granule* and *vacuole* in the reactions of living cells to dyes, so I wish to define as exactly as possible the sense in which I have used these terms. By the term *vitally stainable granule,* I mean particles in the cytoplasm of living cells that take a dye in a uniform manner, and do not increase in size during the functional activity. That is to say, by granules, I mean particles that do not change their reaction to vital dyes nor increase in size during different functional states of the cells. As an example of granules, there are the neutrophilic, eosinophilic and basophilic granules of the leucocytes. It is true that with the Romanovski methods there is some variation of the staining of the neutrophilic granules. Beside these, all of the white cells contain mitochondria in the form of rods that react to Janus green. In the use of the term *granule,* I do not intend to indicate any opinion concerning the physico-chemical state of the substance. On the other hand, by the term *vacuole* I mean certain round droplets of fluid within the cytoplasm that take the stain and also change very greatly in size, according to different functional states of the cells. They may change also in color, provided that the dye used is a chemical indicator like neutral red. These are the digestive vacuoles which Shipley [5] has shown contained phagocytized material. He stained clasmatocytes with neutral red after they had stored trypan blue; he could then see particles of the blue dye within the red vacuoles. Evans and Scott [3] have called these vacuoles the "segregation apparatus" of the cell. They are organs of the cell into which phagocytized material is aggregated. It may not be possible, at the present time, to discriminate completely between granules and vacuoles according to these definitions, indeed, as we come to know more about the functions of the blood-cells, these definitions may be shown to be crude; nevertheless, I believe that at the present time these conventions in the use of terms will contribute to clearness. In the specific instances where I do not know whether a given substance is to be classed with the more constant granules or with the functionally changing vacuoles, I shall use the term *stained particle.* Beside the vacuoles which take a vital dye, there are other clear spots in some cells which do not take a vital dye; they occur in cells which evince signs of degeneration and are, I think, to be considered as evidence of cell-death. Thus, there are two types of structures called vacuoles, first, those that take the vital dyes, and vary with the activity of the cell, and second, those that do not take the stain and can be correllated with degenerative processes.

LEUCOCYTES

1. *Polymorphonuclear neutrophilic leucocytes.*

The neutrophilic leucocytes are very characteristic in these preparations both in their reaction to the stain and in their striking motility. In a successful preparation, they are never still except for the brief second when they stop in order to change their direction. On account of their motility the eye picks them up with great ease in making differential counts. In neutral red, azur or brilliant cresyl blue the neutrophilic granules of human blood-cells stain a uniform faint color. The pseudo-eosinophilic granules of other mammalian forms stain differently. The granules are of equal size and are in constant motion, streaming through the cytoplasm. In Janus green, the neutrophilic granules stain a faint green color while the mitochondria stain a brilliant blue. Unless they are stained with Janus green the mitochondria cannot be discriminated in the leucocytes. Besides these two types of granules in the leucocytes, there may also

be one or two vacuoles in the normal leucocyte. These vacuoles react to the dye more slowly, so that it may be 5 to 15 minutes before they are well stained. They are always larger than the neutrophilic granules and may be even as large as the nucleus. These vacuoles vary greatly in size, in number and in color. They also move in the cytoplasm along with the granules. They can, I think, be used as an evidence of how much phagocytic activity is going on in the leucocytes and, if this conclusion is correct, the presence of these vacuoles indicates that the cells perform a physiological function within the blood-stream. In normal blood there may be none of these vacuoles in the leucocytes; in one case of pneumonia, the vacuoles were very large, even as large as the nuclei, and they had the brilliant scarlet color of the acid reaction of neutral red. In this blood there was a great deal of debris in the plasma. This debris cannot be seen within the vacuoles, for it is colorless, but occasionally a little particle of the jeweler's rouge has been left on the slide and can be seen within the cell. In one of our experimental rabbits, I saw one of the pseudo-eosinophilic leucocytes in which there were four vacuoles, three small ones which had the orange color (neutral reaction of neutral red) while the fourth, a very large vacuole, had a brilliant scarlet color (acid reaction to neutral red).

The type of motility of the leucocytes in these preparations is very striking to hematologists who are familiar only with leucocytes in fixed smears. The normal, living leucocyte is never round and never still. The clear blunt pseudopods are constantly being put out from the cell and the granules stream largely in the direction of the flow of the cell, with, however, many counter currents within the cytoplasm. The nucleus is usually in the rear of the cell and, though it does change in form while the cell is moving, strangely enough it seems to be not as amœboid as the nuclei of the lymphocytes. This, however, may be due to the fact that the nucleus is often obscured by the streaming of the cytoplasmic granules. The type of motility which the leucocytes manifest could only exist in a cell in which the cytoplasm is fluid. In the chick of the second and third days of incubation, I have never seen any cells with true amœboid motion, that is, with motion of great speed associated with marked streaming of granules, except in the case of certain wandering endodermal cells loaded with yolk. The young granulocytes which develop outside of the blood-vessels, and can be seen to wander into them, move but slowly and with very little streaming of granules. They require hours to move a distance which a mature leucocyte could accomplish in minutes or seconds. This point seems to me to be very important with reference to the motility of myelocytes. Myelocytes, like the early granulocytes of the living chick, do not have amœboid motion; they progress only slowly, with slight changes of the form of the cell and without streaming of the granules. Hence we may say that the cytoplasm of the young granulocyte of the embryo or of the myelocyte of adult bone-marrow has a cytoplasm which it too much of a gel to permit of rapid motion. This is interesting in connection with the findings of Chambers[6] that a cell undergoing division has its cytoplasm in the form of a gel. The contrast in motility of the myelocyte and leucocyte of circulating blood is very striking in smears of leukemic blood. The point is also evident if one studies a bit of living bone-marrow with the supravital method, because the leucocytes will wander from the central mass and leave the myelocytes in place. This point is probably of importance with regard to the regulation of the supply of leucocytes to the blood-stream, the myelocytes being usually retained in the marrow.

In watching the living leucocytes, it is of course essential to know the normal rate of motility or rather the normal limits of rate before we can make any judgment concerning variations from the normal. So far I have made only qualitative estimates between active and sluggish leucocytes, calling the cells hyperactive when they move in and out of the field while the count is being made so that it is necessary to skip fields in order to avoid counting cells twice. Dr M. McCutcheon,* of the University of Pennsylvania, has an ingenious method for measuring the rate in μ per minute which must be used in studying deviations from the normal.

The leucocytes are in such rapid motion that no drawing could record their form. Often, in their movement, they put out a pseudopod which becomes a very slender filament, stretching across the entire field and ending in a little ball of cytoplasm at the tip. Such a cell is shown in Fig. 1, though many of these filaments are much finer. Such excessively fine strands of cytoplasm show how the leucocytes can get out between endothelial cells or through the tiniest crevices. Often the ball of cytoplasm at the tip of such a process is lost without seeming to damage the cell at all. These fragmented bits of cytoplasm are very characteristic and are never to be confused with platelets because they always show Brownian movement of the discrete particles. As will be described later, the granules of platelets are in clumps which do not show Brownian movement. Within the cytoplasm of the normal leucocyte, there is, I think, no Brownian movement. The granules do not vibrate but either flow with a steady movement or occasionally dart past others in the cytoplasm. As a cell is beginning to die in these films, especially as the power of locomotion begins to diminish and the cell begins to round up, Brownian motion develops which may mean that the cell has begun to take in too much fluid from the surrounding medium.

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* McCutcheon:—Studies on the locomotion of leucocytes. 1. The normal rate of locomotion of human leucocytes *in vitro*. Studies on the locomotion of leucocytes. 2. The effect of temperature on the rate of locomotion of human leucocytes. Amer. Jour. Physiol., 1923, vol. 66.

In one clinical case (Med. No. 49329) which I studied, there was Brownian motion in the leucocytes while the cell was actually progressing. It was a case of late general carcinoma in which the bone-marrow was markedly involved in the carcinoma as demonstrated by the X-ray photographs. There was another indication that the blood-cells were damaged in this case, namely, they moved very slowly, showing many fine filaments which were often put out and withdrawn without leading to locomotion. Numerous fine filaments of cytoplasm I had often seen in studying living cells in the chicks in artifical media. In 1920, Miss M. A. Herwerden [7] published a method for demonstrating amœboid movement of leucocytes by keeping them in a warm box for half an hour in Deetjen's solution, and then fixing and staining them. In this treatment the leucocytes become completely covered with similar fine filaments as shown in her Fig. 2 on Plate 1. This is, I think, a very interesting reaction, but in my experience it is a sign, not of locomotion but rather of a paralysis of a leucocyte due to an abnormal surrounding fluid. In a moving cell the pseudopods are few in number and much more blunt. At the last meeting of the American Association of Anatomists, held in Chicago in 1923, E. R. and E. L. Clark [8] described the reaction of living amphibian leucocytes after injections of croton oil into the tadpole's tail. They said that the leucocytes wandered from the vessels, and then became stationary, putting out processes so that they looked like "prickle cells." Subsequently they withdrew the processes and became motile again. I have seen such "prickle cells" in studying mammalian leucocytes from marrow in Locke solution; in the one instance in which I have seen human blood after transfusion, the leucocytes were in the form of prickle cells for about an hour. During this time they did not show any locomotion, then they gradually pulled in the processes and began to move again. It may be, then, that the prickle leucocyte is a cell temporarily paralyzed and the observation may be of great value in studying the effect of transfusion on the activity of the leucocytes.

The vital preparations remain in good condition always for an hour, and usually for four or five hours. When the preparation is first made, the leucocytes may round up for a minute or so, but usually they have started to move almost as soon as the oil immersion lens has been adjusted, provided that the box is warm. It is not necessary to warm the slides before making the film. The length of time the slides last makes it perfectly feasible to make differential counts of the cells, though they can perhaps not be made quite as quickly as with fixed smears. In making the differential counts one should record whether the leucocytes move actively or sluggishly, and whether they show the vacuoles that indicate phagocytic activity. Also a tendency to fragment or any other peculiarity of the leucocytes should be noted. In this way we shall gradually accumulate a knowledge of a new set of criteria of the activity or one might even say of the physiology of the leucocytes.

2. *Non-motile leucocytes.*

All of the white cells of the blood take a vital dye except one small group, and these I have called the non-motile leucocytes. In studying smears of normal blood, it soon appeared that there were peculiar, granular forms which neither took up the dye nor showed any motility. Moreover, in these cells the proportion of the nucleus to cytoplasm is not like that of any other granular form, for the nucleus almost completely fills the cell, the cytoplasm being represented by a rim of irregular, refractile granules around the nucleus. Such a cell is shown in Fig. 2. This particular cell has perhaps a more irregular outline than many non-motile leucocytes, because they tend to be round, but the type of outline will bring out the fact that one may not infer amœboid activity from the mere fact of irregularity of contour of a cell. This cell was described by Schilling [9] in 1908, who discovered it in studying living blood-cells with dark-field illumination. He noted that the granules fill the ectoplasm whch is usually free from granules. He regarded them as dead leucocytes and described similar cells, from sputum, which he thought came from very young leucocytes, the nucleus being smaller and directly in the center of the cell. In this paper Schilling also gave some very interesting observations on following the centrosphere in the amœboid leucocytes. He found that in the moving cell the centrosphere maintained its central position regardless of the position of the nucleus. In these non-motile forms he found no centrosphere whatever and thought it was the loss of the centrosome that allowed the nucleus to take the central position.

By studying living smears, I have seen every transition between the non-motile leucocytes and the actively amœboid neutrophilic leucocytes, indicating that, as a leucocyte ages, it stops moving and becomes round; its membrane then becomes impervious to the vital dyes, the nucleus becomes œdematous and the neutrophilic granules change into large, more refractive particles. Schilling calls this process a coagulation of the granules. As will be seen in Fig. 2, these particles vary in size; the nucleus loses all appearance of structure and appears like ground glass. Streaks across the nucleus indicate where the lobes were before the nucleus became œdematous. They may come from cells which had nuclei with three, four or five lobes, rarely from those with nuclei of two lobes.

I tested these cells by letting a drop of Türk's solution run under the coverslip and found that they are retained and so are probably included in total counts of the white cells. It is a possibility that they may have been included in the differential counts with the Ehrlich technique, their cytoplasm having been fixed by heat. Schilling thinks that they show also in Giemsa stain, appearing as cells with a centrally placed, faintly stained round

nucleus, which had been described as modified lymphocytes by Gött and Weidenreich. I think, however, that they are not retained in the technique which involves a fixation in alcohol. I tested them in 33% alcohol, and the granules are dissolved in 13 minutes, while the neutrophilic granules are unchanged. It is difficult to let stronger alcohol run under a coverslip far enough to reach them, since too much coagulation is produced. If they are retained by the Ehrlich technique, this might account for the fact that the Ehrlich data gave a higher percentage of leucocytes, namely 70 to 72 per cent, as contrasted with the more modern data. In 1922 Bunting [10] pointed out this discrepancy from the Ehrlich data and gave the figure 54.6 as the normal percentage of leucocytes from a large group of students from the University of Wisconsin. Corresponding data from the clinical laboratory of the Johns Hopkins Medical School give 65 as the normal percentage.

In the course of some experimental work on the formation of blood-cells in rabbits which Dr. R. S. Cunningham and I are carrying out, it became evident that, though there may be some rhythm in the occurrence of these non-motile cells, there must be exceedingly careful controls concerning their production. The question must certainly be raised as to whether they actually occur in the circulating blood or not, and wherever direct observation of the circulation can be made, an effort should be made to search for them. In the meantime, lacking such complete proof, it has been only possible to eliminate from the technique all factors which can be shown to increase the number of degenerating forms. Among these factors are excessive heat, pressure from the coverslip when the preparation is too thin, and accidental pressure in focusing the microscope. Notwithstanding these points, which indicate that errors in technique produce and increase the number of these forms, the non-motile leucocyte, even when the coverslip presses too heavily, as in the very thin areas, or when the specimen has been overheated, will appear side by side with perfectly active leucocytes, indicating that there are marked differences in fragility in the different leucocytes in our specimens.

Taking every precaution which we have up to the present time analyzed as affecting the production of these cells, namely, securing free flow of blood, counting only films in which the red cells are thickly spread or even overlapping a little, avoiding undue heat, using a regulator in the warm box and maintaining a uniform temperature of 37.5°C., we still find the non-motile cells in our films. We have been making complete counts of the white cells, with differential counts of fixed smears and of the living cells as often as the vital counts can be made, which proves to be about every 40 minutes throughout the day. We make on an average of 12 counts a day. This experiment we have been making with rabbit's blood and have five such series of counts with normal human blood. We find that there are a few non-motile cells scattered through the different counts, or perhaps none, but at one time, usually about the middle of the day, there appears to be a shower of the non-motile forms, which may reach fairly high percentages, as for example 16 per cent or more. When the fixed smears from blood, taken at the same time as the supravital preparations, were counted, it was found that there were six different structures usually regarded as smudges that had to be correlated with the vital counts. First, there are definite, naked nuclei which are either square or rectangular; they have shadows of granules around them and I think correspond to the non-motile leucocytes. Second, there are very long, naked nuclei also with shadows of granules which correspond with degenerating or damaged transitional cells. Third, in making so many counts from the same individual we find small, round nuclei from the small lymphocytes. Fourth, there are compact masses that are pink in Wright's blood-stain that correspond to masses of debris in the living preparations. In the vital counts, these masses of cellular debris, that are so far disintegrated that they cannot be related to the type of cell from which they came, vary on different days from the same individual. These masses are not included in the total counts. In smears they look more like nuclear material than cytoplasmic; in the vital preparations they take none of the vital dyes, so that there is never any question of confusing them with platelets. These masses are being studied by Dr. David T. Smith in connection with lymphatic leukemias of children, in which they occur in large numbers. Fifth, there are the well known masses of fibrin. These are clearly fibrin in the fresh preparations, and in smears they are large pink masses, looking vacuolated and with delicate fibers on their edges. Sixth, there are the so-called fragile leucocytes, in which the cell membrane ruptures while the covers are being drawn apart. These fragile leucocytes in which the scattered and stained granules make it perfectly clear from what cell they came, whether neutrophilic or eosinophilic, are usually disregarded in making differential counts. There is no doubt but that they are mechanically increased by faulty technique, by dust on the coverslip, or by an uneven sliding apart of the two coverslips. They never occur in the living preparations, where there is no dragging of the cells across the film, nevertheless, in correlating the counts of fixed smears with vital counts they must be included. They can be shown to have been represented in a given double count by actively motile forms in the living state. On the other hand, in one of our series the fragile leucocytes had to be added to the naked nuclei of the fixed smear to give any correlation with the count of the non-motile leucocytes in the fresh specimen. Such preparations show that there is the same difficulty, in analyzing the fragile leucocytes in smears as the non-motile forms in the living, to discriminate how much is

due to technique and how much to the condition of the original cell. It may be stated that fragile leucocytes may be produced at any time by rough handling, but that when the leucocytes are about to go into the non-motile phase, they may appear as the fragile forms in the fixed smears.

In the series from human blood, there is also some indication of a period during the day when there is a considerable increase in the proportion of the small lymphocytes which we regard as the old form. I am well aware that this report of the non-motile forms and of the aging of lymphocytes is incomplete, indeed quite fragmentary, and I hope subsequently to give an analysis of the daily cycle of the white cells with a more standardized technique and with more adequate data. It would then be possible to give a curve of the rhythm of the types of white cells. I mention these experiments at this time, however, because in the study of blood in clinical cases, there are often very marked discrepancies which are usually disregarded. For example, there are often smears of blood with great numbers of smudges which cannot be repeated a few hours later. In our experience the actual showers of the non-motile form last only a half hour or so, which would easily account for such cases.

The significance of the non-motile cells, if we can prove that they do occur in the circulating blood, seems to me to be that they give us a chance for the first time to estimate the normal death rate of the leucocytes, indeed to study the normal rhythm of the blood-cells. In the studies made by Arneth [11] on the number of lobes of the nuclei of the leucocytes, there was given an indication of the relative age of the leucocytes to be found in the circulating blood, but we have never had any way of estimating the number that were dying. These non-motile cells seem to me to show that there is a real chemical transformation of the cytoplasm as the cell dies, so that they should be studied from the standpoint of whether they give a substance to the plasma which has to do with maintaining the normal rhythm of the leucocytes. If we can secure such cells; the point might be subjected to experiment. In this connection, the cells of pus must be studied with the vital method. It is obvious that we need to know the mechanism of maintaining the normal rhythm of the different forms of the white cells as a preliminary for studying the causes of variation from the normal and it is certainly a rational idea that the normal death rate might be correlated with this rhythm, that is, that the dying cells might produce the substances that would attract similar cells into the blood-stream. Here it is obvious that there are two factors to be analyzed, the actual production of new leucocytes in the marrow and the calling of them into the blood-stream. The maintenance of the normal rhythm is certainly complex; when large numbers of cells, leucocytes or lymphocytes, have been called from the blood into the tissues, the factors are even more complex, and to analyze this mechanism is now one of the major problems connected with the blood.

3. *Basophilic leucocytes.*

The method of studying blood-cells in the living form disproves conclusively the idea of Weidenreich,[12,13,14] that the human basophilic leucocytes of the blood-stream are degenerating cells. They are unquestionably living cells, as has been maintained by Naegeli [15] and by Bunting.[10] Of course it will now be possible to find the changes in the basophilic leucocytes of the blood-stream that indicate cell death, but so far I have seen only living basophilic cells. Some of them have nuclei that are slightly polymorphic but even the ones with the peculiar rosette-shaped nuclei, such as are shown in Weidenreich's [12,13] figure, are living cells, as proved by the fact that the nuclei do not stain in vital dyes and that the cells themselves are amœboid. In human blood the basophilic granules are intermediate in size between the neutrophilic and the eosinophilic granules and they have the special characteristic of taking the dye unevenly, some being much darker than others. The granules are all round but not all of the same size as are those of the neutrophilic and eosinophilic types. They give a deeper red reaction in neutral red than either of the other forms.

In the motility of the basophilic cells of human blood there is but little streaming of the granules and their rate of motion is much less than that of either of the other two groups of the leucocytes. I have not seen any changes in these cells to be related to function, that is to say, I have not analyzed any of the stained particles as vacuoles. The observations on them have been, of course, limited on account of their small numbers. With the supravital methods the relations of the basophilic cells of the blood to those of the marrow and of the diffuse connective tissues can now be attacked again.

4. *Eosinophilic leucocytes.*

The eosinophilic leucocytes have the large granules which take the dye uniformly and more intensely than the granules of the neutrophilic leucocytes. Like the neutrophilic cells, they show an active amœboid motion with a streaming of the granules. I have never seen them remain motile as long as the neutrophilic cells, but I have seen them move as fast. Usually they move at a slower rate and do not remain in motion in the preparations more than half an hour. I have never made out any vacuoles in the eosinophilic cells. So far I have not studied any cases in which there was a marked eosinophilia. The nuclei are usually two-lobed. When the cell moves, the two lobes may be dragged far apart, a slender thread keeping them from separating. The two lobes change in form as the cell moves, but not as much as the nuclei of the lymphocytes.

5. *Monocytes.*

In studying the living blastoderms of chicks on the third day of incubation, I [16] found that there is one group of the white cells that comes from endothelium. On the

second day of incubation, the origin of the red cells from the endothelium of the vessels can be seen with great clearness. On the second day, all of the single cells or clumps of cells clinging to the inner lining of the vessels of the area vasculosa can be seen to contain hemoglobin by the color of the living cell. The amount of the hemoglobin may be too small to show in the deeply basophilic cytoplasm of the fixed cell, but the yellow tinge is evident in the living state. On the third day, an occasional cell becomes free from the inner wall of the vessel which not only does not show hemoglobin, but does show the stained particles and the stained vacuoles that characterize the monocyte stem. These cells I think are analogous to the monocytes of the adult blood. At the same time, for the next few days of incubation, very large masses of similar cells wander from the outer surface of the endothelium of the vessels of the area vasculosa. These are the clasmatocytes. Aschoff and Kiyono [17] believe that the clasmatocytes of the connective tissues and the monocytes of the blood should be grouped together under the common name of histiocytes.

In studying normal, human blood, it becomes evident that there are two strikingly different types of cells in the blood belonging to the monocytic strain. Indeed they are so different in appearance as to require some proof that they are only two phases in the life of a single cell. The first form corresponds to the large mononuclear form of Ehrlich, a cell entirely different from the large lymphocyte, the second is the transitional cell of the Ehrlich classification.

The large mononuclear cell is shown in Fig. 3. It is a large cell, almost always round, varying in size from a cell slightly larger than a leucocyte to one the size of a myelocyte. The nucleus is usually round, may be slightly indented, and is always eccentric. The most striking characteristic of the cell is that its cytoplasm is completely filled with fine uniform particles that stain slightly darker than the neutrophilic granules in neutral red. The particles of the cells are usually still, or they may be in slight motion. In the very young forms, a spot free from granules in the center of the cell opposite the nucleus indicates the centrosphere. The cell shows no active locomotion, but it may move very slowly so that by watching it 10 to 15 minutes one may perceive a change. After one is familiar with this cell with the vital dye, it can be distinguished without any stain at all, that is, the same granules are very plain in the living cell. It could never be mistaken for the large lymphocyte, as can be seen by comparing Figs. 3 and 7. The cell is to be distinguished from a neutrophilic myelocyte by the fact that the particles are smaller than the neutrophilic granules and have a slightly different shade of color in neutral red. The two cells may be found side by side in bone-marrow and compared. There are, however, but few large mononuclear cells in bone-marrow. It will be noticed that I have used the term "particle," in connection with this cell, advisedly, because so far it has not been possible to determine the nature of the substance in the terms of my definitions.

The transitional cell, on the other hand, varies much more in size than the large mononuclear just described, though the typical ones are very large. They are shown in Figs. 4 and 5. In the living preparations, they are seldom round, in fact they are usually very long, but may be quite irregular, as in Fig. 4. They show considerably more motility than the large mononuclear form and the particles in the cytoplasm are often in quite active motion. The locomotion of the cell is slow and not as correlated with a flowing of granules as is the case of the leucocytes. The cell is especially conspicuous for the amount and the variety of the substances stainable with vital dyes. Its cytoplasm is filled with particles stainable with neutral red, which vary in size all the way from a fine dust to huge vacuoles. At present I do not know the relationship between the particles and the vacuoles, but when the cell is highly stimulated to phagocytic activity so that its cytoplasm is filled with large vacuoles, as in Fig. 5, the fine particles have disappeared. The fine particles also stain in Janus green, giving a greenish tinge to the cell in which can be seen small, scattered mitochondria. Perhaps it is the same fine particles that give the cytoplasm its color in fixed smears. Of all the cells of the circulating blood the transitionals change the most with function, the large mononuclears the least. The only change I have seen in the latter is a slight increase in the motility of its cytoplasmic particles while in the case of the former, if my interpretation of the stainable vacuoles is correct, the transitional is the most phagocytic of all of the cells in the blood stream; that is to say, a single cell can phagocytize a greater amount of substance than any other cell. They vary all the way from a cell filled with fine particles and no vacuoles to a cell whose cytoplasm is so loaded with vacuoles that nothing else is visible.

These two cells are so strikingly different in the living state, that it would be impossible from normal blood to conclude that they both belong to the same group, namely, the monocytes. But I had the opportunity to study one case of abnormal blood in which it became clear that they are merely two phases in the life cycle of the same cell. This very interesting case was one of Malta fever. The patient was admitted to the Johns Hopkins Hospital October 18, 1922, (Med. No. 48513) and showed a fever of a moderate grade correlated with a blood-count in which the total number of the white cells was normal but there were ten per cent of monocytes. The diagnosis of Malta fever was made by Dr. Amoss by finding the organism in the circulating blood. He made an autovaccine and gave it in four doses at intervals of four and five days. The first vital differential count which I made on November 25th was as follows:

Polymorphonuclear neutrophiles	32%
Non-motile leucocytes	6.2
Eosinophiles	1
Basophiles	1
Lymphocytes	39.5
Large mononuclears	.7
Transitionals	18.5

In actual percentage the count did not differ much from this throughout the course of the disease, the monocytes remaining about twenty per cent. After the second dose all of the monocytes were of the transitional form; after the third dose there was a very marked increase in the stainable substance of the transitional cells, the cytoplasm being completely filled with large stained vacuoles. As a sign that they were actively phagocytic, red blood-cells were found engulfed in them. After the fourth dose there was a brilliant demonstration of the fact that the transitionals are merely older, more active large mononuclear forms. There were a few large mononuclear forms of a young stage, that is, the centrosphere was plainer in them than I have ever found it in these cells. From these young forms there was every transition from the mononuclear to the transitional form, of the inactive and active state, on to cells that were degenerating. No sharp line could be drawn between the large mononuclear or young forms and the transitional. Dying cells have very little reaction to the vital dye, show no movement of particles, no locomotion and have clear unstained vacuoles in the cytoplasm. The entire life cycle of the monocytic strain from the youngest to the dying cells could be seen in one preparation.

The subsequent history of the case was also interesting. I had a chance to follow the case long after the acute illness was over. The leucocytes remaining very low, the increase in monocytes being at the expense of the leucocytes, Dr. Amoss gave the patient a series of three doses of typhoid vaccine, in the hope that there might be an increase in the percentage of leucocytes. Instead, there followed a stimulation of the lymphocytic strain of cells. Lymphocytes showed a marked increase in the number and size of the vacuoles in the neutral red and there was a marked variation in their staining reaction in Wright's stain. This gradually subsided but the increased vacuolization of the monocytes continued over a period of some months. Gradually this subsided. The patient was discharged from the hospital with a negative blood culture on December 10, 1922, but it was not until April that the staining reaction of the monocytes became normal. On April 24, 1923, the monocytes were still ten per cent of the white cells, but they were normal in staining reaction. Blood cultures were still negative.

From the study of this case I became entirely convinced that the large mononuclear cell is the younger stage of the transitional form. The transitional form is more active as shown both by increased motility and by increased phagocytic power as indicated by the large numbers of stainable vacuoles.

In connection with these studies of blood, I have been making smears of living cells from lymph glands and from the spleen in different animals. I press out a little fluid from the gland, put it on a slide prepared as for the blood films and then add a small amount of a scraping from a freshly cut surface. In such preparations, as is well known, there are always many clasmatocytes, that is, cells of the transitional type from lymph glands, spleen and bone-marrow, but the cells identical in type with the large mononuclear of the blood stream I have found in any considerable numbers only in scrapings from the spleen. The animals used have been cats and rabbits.

6. *Lymphocytes.*

The lymphocytes have proved to be the most difficult of the white cells to analyze. As a matter of fact we have had no convincing proof as to which is the young form, one group of hematologists thinking that it is the large lymphocyte, the other that it is the small. I am confident that these vital studies offer very considerable evidence toward the view that the large lymphocyte is the young stage. As was shown by Miss Simpson, lymphocytes are characterized by definite clumps of large mitochondria. All of the lymphocytes, with one occasional exception, have a cytoplasm that is strikingly clear in the living form. This is in marked contrast to the fine granulaton of the monocytes. In the cytoplasm of all lymphocytes there are two substances that take vital dyes; first, they all have clumps of mitochondria, and second, two or more small vacuoles reacting to neutral red. In Fig. 7, is the large lymphocyte, showing three vacuoles and the characteristic mitochondria, placed as usual opposite the nucleus. Both of the substances are often in motion and an occasional rod or vacuole is to be found along the rim of the nucleus. The large lymphocyte is almost always rounded up, as shown in this figure; its nucleus is round or oval, seldom irregular and the cell very seldom shows any locomotion. The striking characteristic of the intermediate form is that it is frequently in motion. Also it shows much more variation in the reaction to neutral red. Such a cell is shown in Fig. 6. The intermediate cell is distinctly smaller than the large lymphocyte; it has the same cluster of mitochondria opposite an eccentric nucleus. Among the rods are a few vacuoles of which several are shown in Fig. 6. The cell moves much more slowly than the leucocyte; when the cell is moving, the nucleus is usually in the front end and its form is constantly changing. Both the mitochondria and the vacuoles are in active motion, not in the least correlated with the locomotion of the cell; indeed the particles may be in very active motion when the cell itself is quite still. The particles stainable with neutral red in the lymphocytes vary from one or two up to about ten or even more. The small lymphocytes al-

ways show the same clump of mitochondria, and at least one or two vacuoles. The vacuoles may increase in size and number but less markedly than in the intermediate forms. The small cell seldom moves at all.

In all of the lymphocytes the cytoplasm is much more clear than that of the monocytes; that is to say, the lymphocyte lacks the fine granulation of the monocyte. Indeed it is on this account that the mitochondria are very readily seen in the living cell without being stained at all. This is not true of any of the other cells of the blood. There seems to me to be little doubt but that the substances that stain in neutral red in the lymphocytes belong to the category of vacuoles in my definition; that is to say, they are structures that vary markedly in different functional states of the cell. This can often be made out very clearly in the case of the small lymphocyte where the two or three vacuoles can become markedly larger than usual without causing the least difficulty in discriminating the cell from all other types in the blood. The size of the cell is so distinctive and the massive blotches of chromatin in the nucleus are so plainly seen in the living state that the cell cannot be confused with any other. With the intermediate and the large lymphocyte the case is different. When they are highly stimulated, the neutral red vacuoles may approach in size and number the condition of the monocytes. The mitochondria remain the same and it is then necessary to use the double stain vitally and to correlate the differential counts with studies of fixed smears.

There is one form of the lymphocyte which occurs occasionally in normal blood in which the nucleus is exactly in the center of the cell instead of having the usual eccentric position, and in these cells, which belong to the large and intermediate group, the cytoplasm shows a very fine granulation like ground glass. In the fixed smears these are the cells with markedly basophilic cytoplasm. I think that they will be understood only when we have a complete account of the life cycle of the lymphocytes.

In studying lymphocytes in the living forms, it is striking how seldom the nuclei are as round as in the fixed smears. This is true even of the small forms. In cases of lymphoid leukæmia, I have found marked changes in the nuclei, especially of the small forms. In one case (Med. No. 49241) a large proportion of the small lymphocytes had nuclei which were split in half or fragmented into three parts. While this was very clear in the living specimens, fixed smears did not show these degenerations of the nuclei at all. In this case I saw a nucleus of a large lymphocyte divide by direct division, division of the cell did not follow but there was a division of the mitochondria into two parts opposite the cleft in the nucleus. Subsequently I found two more large lymphocytes with two nuclei and with the same arrangement of mitochondria. All of these observations indicated unusual or pathological conditions of the lymphocytes and it was very striking in this case that there was no increase in phagocytic power, that is to say, there were almost no vacuoles in neutral red in the entire strain of the lymphocytes in this case. Thus there were marked changes in the nuclei of the cells correlated with low functional activity.

It has been striking that the lymphocytes taken directly from the lymph glands have not shown as many or as large vacuoles as those of the blood. I do not know whether there is any correlation to be made between the azurophilic granules and the vacuoles that stain in neutral red, but I think that there is no such change in the size of the azurophilic granules as there is with the vacuoles.

There was a patient in the Johns Hopkins Hospital (Med. No. 489984, Path. No. 7383), whose case was very interesting in connection with the lymphocytes. The condition was exceedingly complex; no definite diagnosis could be made even from the autopsy, but it was probably related to the lymphoid leukemias. The patient was in the hospital during the last month of life. The striking point in the case was the enormous enlargement of spleen and lymph glands. Until the last few days the total count of the white cells was normal and the differential count showed only signs of the so-called pathological lymphocytes. Finally, there was a marked leucocytosis reaching as high as 110,000 cells, during which strangely enough the proportions of the cells were still approximately normal. Myelocytes appeared in the blood-stream during the last four days of life and at autopsy the bone-marrow was found to be active.

I had an opportunity to study smears from the lymph glands, spleen and bone-marrow from this case while the cells were still living. Similar smears from the lymph glands and spleen of cats and rabbits show groups of cells, stuck together in clumps, which are in no sense syncytial masses, since the cells can be separated by pressure. These cells have the following characteristics: the cytoplasm is as basophilic as the nucleus; the nuclei are the least visible of any living nuclei I have yet worked with. In smears with Wright's blood stain, the cytoplasm of these cells is almost as basophilic as the nuclei. In the living state there are no granules of any sort to be made out in these cells. This type of cell may well be the stem cell of the blood-cells that do not come from endothelium, namely, of the leucocytes and lymphocytes. In this case in which the striking changes were in the lymph glands and spleen, the spleen had similar masses except that every cell had a clump of mitochondria like those that characterize lymphocytes. This may represent a differentiation of the primitive cell into a forerunner of the lymphocytic series.

From these studies, it is my opinion that the large lymphocyte is the young form of which only a few occur in the normal blood; that they are comparable to the myelocytes of the granulocytic strain except for the fact

that a few do occur in the blood. The intermediate forms are, I think, more active functionally, first, because they show the greatest amount of motility, and second, the greatest variations in phagocytic power. The small forms are the oldest, but most of them are active cells with power of phagocytosis. They show the greatest number of degenerating forms. The only suggestion which I have concerning the cells with marked increase of the basophilic reaction of the cytoplasm is that they may be near the phase of cell division, in which there is a general increase in basophilic reaction of cytoplasm.

It has already been indicated that there are some difficulties in analyzing the blood-cells in the vital method. The most frequent difficulty is the one just mentioned, that of discriminating lymphocytes, when they are in a state of unusual phagocytic activity, from moderately active monocytes. In such cases comparison must be made with fixed smears and the case must be followed over a period of time. In other instances, as for example in certain cases of infectious mononucleosis, I think that it is possible to say definitely that it is the lymphocytes that cause the increase in mononuclear forms and not the monocytes. Another difficulty, I have found, is the distinguishing of certain very sluggish leucocytes, in which there has been a marked depletion of the neutrophilic granules, from inactive monocytes. As with all other methods, there are from time to time cells which must be regarded as unclassified.

7. *Platelets and Megalokaryocytes.*

Platelets are very characteristic in the vital dyes. They have two types of granules, particles that occur in clumps and stain with neutral red and discrete particles that stain blue in Janus green. These particles are always still, never in Brownian motion. These characteristics distinguish them from all other forms of debris in vital preparations. In these vital films platelets are more often found in large clumps than they are in fixed smears.

In a case of chronic myeloid leukemia (Med. No. 48856) admitted on November 25, 1922, we were surprised to find very large numbers of megalokaryocytes in the circulating blood. In this case there were 80,240 white cells on November 26th. On November 28th I counted 306 white blood-cells and 19 megalokaryocytes, making 325 cells counted. Besides these there were 18 large masses of platelets without nuclei. Thus the nucleated giant cells made approximately five per cent of the total white cells in the blood. The next total white count was 89,280, made on March 5th. Estimating the number of white cells on November 28th as 80,000, there were then 4000 megalokaryocytes per cu. mm., in the circulating blood. In studying these giant cells on the slide, the granules stainable in neutral red gradually formed in little clumps and the cytoplasm then fragmented into typical platelets on the slide. In Fig. 8 is a typical giant cell as it appeared on November 28th. In Fig. 9 is one with very little cytoplasm but with a nucleus very typical of the megalokaryocyte to be found in the bone-marrow and spleen. On March 4th, the patient was given a dose of X-rays, and two days later the total number of the white cells dropped to 55,350 and all the giant cells had disappeared from the blood.

In Fig. 10 is a cell taken on March 4th, in which I followed the disintegration of the outer mass of cytoplasm into platelets. As will be seen, the nucleus with a very small amount of unfragmented cytoplasm is in the upper left hand corner of the mass, most of the cytoplasm being in the form of platelets. The contents of some of the platelets were not drawn, since the cell became too disintegrated to show their character. These observations seem to me to be a confirmation of Wright's theory that it is the giant cell which gives rise to platelets. Certainly platelets can be definitely separated from the debris of the blood-cells in the vital preparations. Since, according to Minot,[19] the occurrence of giant cells in the blood stream is not as uncommon as had been heretofore supposed, the number of cases identified will I am sure increase where the technique of studying blood supravitally is introduced, since the cells are so much more easily identified in these preparations than in stained smears where the giant cells are almost certainly damaged. Moreover, the occurrence of any very large clumps of platelets in the circulating blood will suggest that giant cells be searched for.

In conclusion, in these studies of white blood cells, it has been shown that there is much evidence to favor the view that the large mononuclear cell is the young form of the monocytic strain and that the large lymphocyte is the young stage of the lymphocytes. The method offers a chance to distinguish cells which have been stimulated from those which are degenerating. I am convinced that the method opens a new phase of the physiology of the white blood-cells. The next step will be to complete the life cycle of each type of white cell. With these data in hand we shall be in position to complete the study of the normal rhythm of the leucocytes and thus have a foundation for analyzing the method for maintaing this rhythm.

LEGENDS

Fig. 1—Polymorphonuclear neutrophilic leucocyte from normal, human blood, drawn from the living cell in which the neutrophilic granules have been supravitally stained with neutral red. This is a free-hand drawing; the motion of the cell was too great for the use of a camera lucida. It shows the general form of an advancing pseudopod becoming a long filament. 1a is a red blood-cell for the magnification.

Fig. 2.—Non-motile leucocyte from normal human blood. The preparation from which this cell was taken was supravitally stained with neutral red, but this cell did not take any of the dye. The large mass in the center is the nucleus, which had four lobes before it became œdematous. The irregular granules which fill both the endoplasm and the ectoplasm were highly refractive. 2a is a red blood-cell for the magnification.

Fig. 3.—Monocyte of the large mononuclear type, from normal human blood, stained supravitally with neutral red. The magnification is not given, but it is the largest type in the circulating blood.

Fig. 4.—Monocyte of the transitional type, from normal human blood, stained supravitally with neutral red. The cell was in motion; in the cytoplasm are two types of substance that reacted to the dye, fine particles and larger, round vacuoles. 4a is a red blood-cell for the magnification.

Fig. 5.—Monocyte of the transitional type, taken from the blood of a patient after an attack of Malta fever (Med. No. 48513), but while the monocytes still showed a marked stimulation to the formation of vacuoles. The vacuoles, which were stained in neutral red, are shown in gray. This is the same type of cell as that shown in Fig. 4, except that it was more active functionally. Drawn with a camera lucida, Leitz Obj. 1/12, Zeiss Comp. Oc. vi, and then enlarged 3×.

Fig. 6.—Lymphocyte from normal human blood, supravitally stained with neutral red. The cell was moving in the direction of the arrow. In the cytoplasm are 21 vacuoles which were stained, and and a clump of mitochondria in the form of rods which were not stained. The nucleus is markedly irregular in consequence of the movement of the cell. 6a is a red blood-cell for the magnification.

Fig. 7.—Lymphocyte of the large type, from normal human blood, supravitally stained with neutral red and Janus green. It shows the characteristic clear cytoplasm of the lymphocytic strain. The nucleus is eccentric; in the main mass of the cytoplasm are four small vacuoles which stained with neutral red and a large clump of mitochondria which stained in Janus green.

Fig. 8.—Megalokaryocyte from human blood, taken from a case of myeloid leukemia (Med. No. 48856). The nucleus is in the upper part of the cell and the cytoplasm shows a marked granulation which was supravitally stained in neutral red. The character of the cytoplasm was accurately copied with the camera lucida. (Leitz Obj. 1/12, Zeiss Comp. Oc. vi.)

Fig. 9.—Megalokaryocyte drawn from the same preparation as the cell of Fig. 8. It shows a cell which has lost most of its cytoplasm; the nucleus is mulberry-shaped. 9a is a red blood-cell which corresponds both to this figure and to Figure 8.

Fig. 10.—Megalokaryocyte from the same case as Figs. 8 and 9. This preparation was taken from the patient on the morning of December 4th, immediately before he received the first dose of X-rays. The slide was given the same exposure to the X-rays as the patient. The giant cells were watched throughout the day for the disintegration of the cytoplasm into platelets. The preparation was kept in the incubator over night and the drawing was begun on the next day, the leucocytes being still alive and moving. On the next day, Dec. 6, an attempt was made to complete the drawing and the dead cell marked 10c was put in. The character of the platelets, however, had changed so they were left with the outlines originally drawn with the camera lucida. On this day all of these cells disappeared from the blood of the patient. 10a is a red blood-cell; 10b is the nucleus of the giant cell. (Leitz Obj. 1/12, Zeiss Comp. Oc. vi.)

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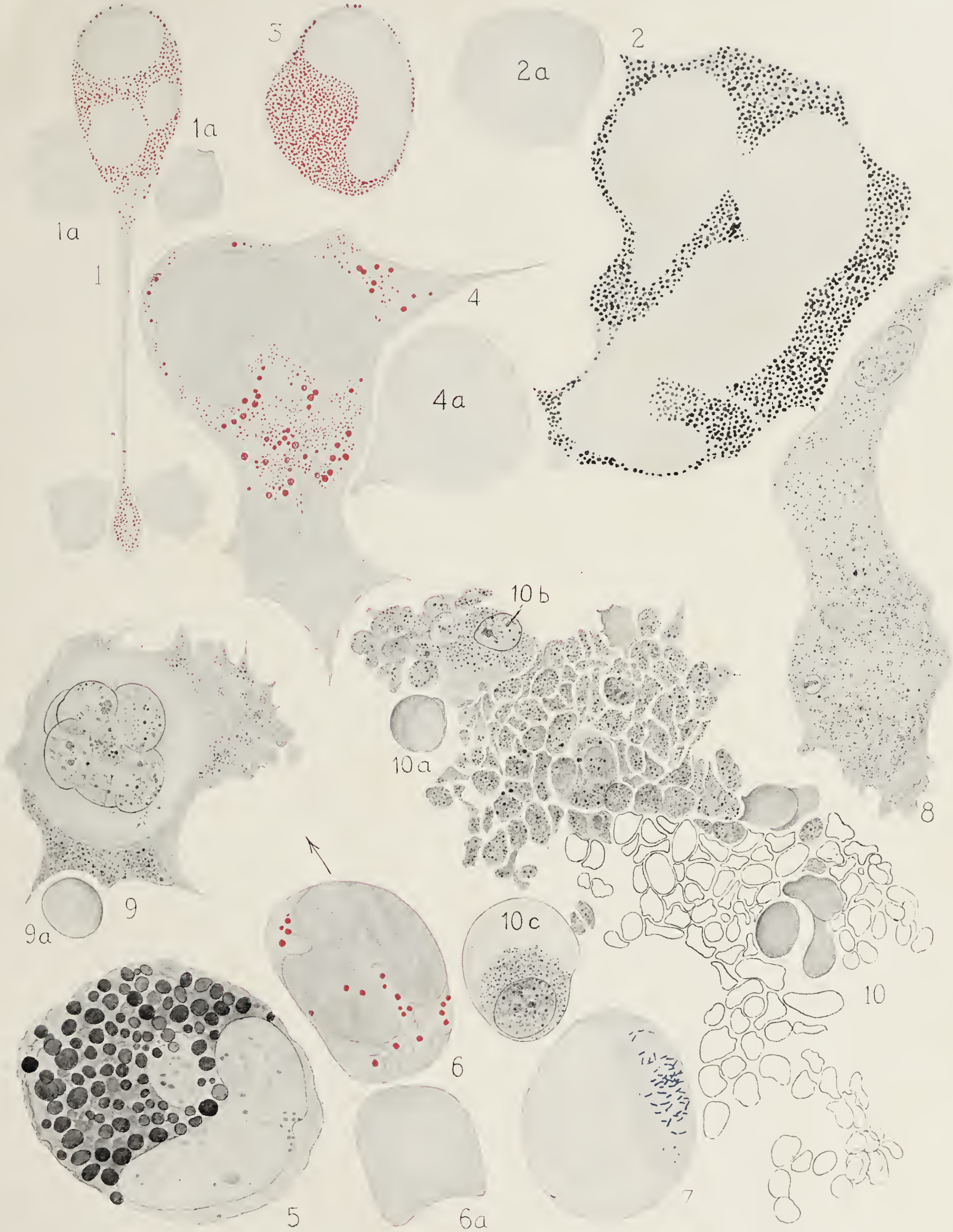
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THE PATHOLOGICAL ANATOMY OF THE CORPUS LUTEUM

(ABSCESS, CYST, HEMATOMA, AND NEOPLASM)

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INTRODUCTORY

Much of the remarkable advance which the past two decades have shown in our knowledge of the physiology of the female reproductive organs has come through the study of the life history of the corpus luteum. The cause and mechanism of menstruation, its relation to ovulation, the menstrual histology of the endometrium, the influence upon both menstruation and ovulation of the various incretory glands—these are only a few of the problems which have been so assiduously and so profitably studied since the establishment of our newer concepts of the menstrual phenomenon. The histological development of the corpus luteum has been studied by many investigators. Certain aspects of the problem appear to have been conclusively studied; others are still obscure.

Relatively little seems to be known, however, concerning the pathological anatomy of the corpus luteum, and the influence of abnormalities of lutein tissue upon the functions of the reproductive organs, especially menstruation. It is true that a few scattered efforts have been made to correlate quantitative variations in the lutein tissue of the ovary with variations in the amount of menstrual bleeding (de Rouville and Sappey, Novak). These studies can not be considered very conclusive, chiefly because of the still unavoidable necessity of using the histological appearance of a tissue as the basis for conclusions as to its functional importance—an obviously risky procedure.

In a general way, the pathological changes which may implicate the corpus luteum are (1) the inflammations, manifesting themselves practically always as abscesses; (2) the cysts; (3) the hematomas; and (4) neoplasms. Inflammatory involvement of the corpus luteum is very common, but is practically always only an incident in a more or less extensive pelvic inflammatory process. The cysts and the hematomas are the two types of lesions which lend themselves most readily to investigation, and the study of which, theoretically at least, might be expected to throw most light on the pathologic physiology of the corpus luteum. Neoplasms have been described by various authors, as we shall see, but their occurrence is very rare.

The literature contains quite a number of studies upon the various pathological changes to which the corpus luteum is subject. There is, however, such a wide variation in the individual point of view of different authors, that one is apt to become confused when one attempts to correlate these studies. This is particularly true because many of these investigations were carried out before the beginning of our newer knowledge concerning the life cycle of the normal corpus luteum, so that the conclusions arrived at by the authors must be adjusted to the present day point of view. For such reasons as this we have thought that a brief résumé in one paper of all the more important pathologic changes which may affect the corpus luteum should be of interest to all gynecologists, especially those who have studied the pathological aspect of the subject. We have, therefore, gone over the pathological material in the laboratory of Gynecological Pathology of the Johns Hopkins Hospital for the past three or four years, and have in this way, we believe, secured an adequate basis for a scheme of classification of the principal structural alterations which may be noted in the corpus luteum. This classification, as will be noted, takes as its point of departure the physiological variations exhibited by the corpus luteum, particularly as related to the various phases of the menstrual cycle.

Many hundreds of sections have been studied in going over our material. Certain lesions of the corpus luteum have been encountered rather frequently; others, again, are quite rare. The accompanying table presents, in compact form, the division of the subject which has suggested itself to us, and which, we trust, may prove of service to other workers in gynecological pathology.

PHYSIOLOGICAL AND PATHOLOGICAL VARIATIONS OF THE CORPUS LUTEUM

I. The normal cycle.
   - (a) Stage of proliferation.
   - (b) Stage of vascularization.
   - (c) Stage of maturity.
   - (d) Stage of retrogression.

II. Physiological variations.
   - (a) Cystic form.
   - (b) Hemorrhagic form.
   - (c) Corpus luteum of pregnancy (cystic and solid).

III. Pathological variations.
   - (a) Corpus luteum abscess.
   - (b) Corpus luteum cyst: (1) recent, (2) old.
   - (c) Corpus luteum hematoma: (1) recent, (2) old.
   - (d) Corpus albicans cyst.
   - (e) Corpus albicans hematoma.
   - (f) Combined types.
   - (g) Corpus luteum neoplasm (luteoma).

IV. Allied conditions—Multiple "lutein" cysts of the ovary.

Cyclic Changes in the Corpus Luteum

As a preliminary to a discussion of the abnormalities involving the corpus luteum, it may not be inappropriate to present at least a sketchy review of the normal cyclical variations which this structure exhibits.

The earliest stage in the life cycle of the corpus luteum, (the stage of *proliferation or hyperemia,* Figs. 1, 2) is characterized by a beginning lutein-like transformation of the granulosa cells, although the theca cells at this stage bear a far more striking resemblance to genuine lutein elements. The corpus luteum in this phase is an inconspicuous flattened vesicle, with a grayish wall. Hemorrhage into the lumen may occur at times, but is not characteristic until the next stage, that of *vascularization* (Fig. 3). During the latter the lutein transformation of the granulosa cells becomes much more advanced, and the lutein layer is invaded by newly formed capillaries pushing in from the theca. Hemorrhage into the lumen is the rule, usually in the form of a narrow, hemorrhagic zone to the lumen side of the lutein layer. This stage is of importance in the discussion of corpus luteum hematomas, for the latter commonly arise, at this stage, from an excessive hemorrhage which inundates the cavity of the corpus, as will be discussed later.

Following the stage of vascularization comes that of full *maturity,* (Fig. 4), which reaches its acme just before the onset of menstruation. The corpus luteum now appears as the large, yellow body of tradition, with a broad, festooned, brilliant yellow wall made up of well formed lutein cells. The lutein zone is marked off from the lumen by a limiting layer of newly formed connective tissue, which apparently differs in extent in different corpora lutea of the same chronological period.

An important stage of the corpus luteum life cycle, from our present point of view, is that of *retrogression,* (Fig. 5), which begins at the onset of menstruation. There is a gradual shrinkage of the lutein layer, together with fatty degeneration of the lutein cells. This fatty change, according to Miller, is found in lutein cells only after the beginning of retrogression. The cells soon become atrophic, while pyknosis and nuclear fragmentation are conspicuous. Even more striking is the gradually advancing fibrosis proceeding from the theca through the lutein zone and into the connective tissue of the cavity. Hyalinization of this fibrous tissue is noted almost from the beginning, until soon the entire lutein zone is converted into a festooned mass of hyaline tissue, sharply walled off from the ovarian stroma externally, and usually from the connective-tissue core within the lumen. Miller emphasizes the fact that this hyaline change involves the connective-tissue scaffolding of the lutein layer rather than the lutein cells themselves. The *corpus albicans* (Fig. 6) thus produced is quite different in appearance and structure from the corpus fibrosum, which represents the end-product of the process of atresia folliculi.

Physiological Variations in the Corpus Luteum

Cystic forms.—The study of both cysts and hematomas of the corpus luteum is made difficult by the fact that many normal corpora lutea show a greater or less degree of cystic distention of the lumen, and that all corpora lutea show at certain stages some degree of hemorrhage into the lumen, which may at times be sufficient to stamp them clinically as hematomas. It is difficult to draw a line between what is normal and what is abnormal from both these points of view. Few will agree with the assertion of Santi that all cystic corpora lutea are to be looked upon as abnormal, because the cystic change is contrary to the innate tendency of the corpus to contract and shrivel up. Cohn says that frequently the central fluid content of a normal corpus is several times as great in diameter as the lutein layer, perhaps even four to eight times as wide. And yet it is certain that many of these large cystic corpora have been described as pathological cysts. Martin, many years ago, asserted that one could speak of cysts only when such structures reach a size larger than that of the ovary.

It is the character of the cyst wall which must, in large measure, be depended upon to distinguish between merely cystic but functionally active corpora lutea on the one hand, and, on the other hand, pathological corpus luteum cysts which have dropped out of the physiological cycle of the ovary. In some cases this distinction is very easy, as where the lutein layer is markedly retrogressive and fibrotic, and where it is buried beneath a heavy layer of organized connective tissue. In other cases this point is far more difficult to settle. The lutein layer may be covered on the lumen side with only a light connective-tissue deposit, and the evidences of retrogressive change may be very uncertain. Furthermore, as has already been emphasized, it is always dangerous to draw conclusions as to the functional activity of a tissue from the histological appearance alone. If the investigations of Miller are accepted, much importance should be attached to the presence of fatty changes in the lutein layer.

Hemorrhagic forms (Fig. 7).—The same general considerations would seem to apply to hematomas of the corpus luteum. These, as has already been indicated, are often of no pathological significance. The amount of bleeding seen in normal corpora lutea differs in different species. It is said to be constant and rather free in pigs and horses, whereas in rabbits it is rare to see much bleeding (W. L. Williams). In the human being there is always some bleeding in the stage of vascularization, although it may be so slight as to cause only a thin zonal layer along the inner margin of the lutein cells. To show the great frequency of ovarian hemorrhage in general, mention may be made of the recently reported studies of Runge. This investigator cut up the ovaries

from 120 cases, in sections 5 mm. thick, and found that only a very small number (14) showed no hemorrhage at all, and in these cases the ovaries were senile and atrophic, without follicles or corpora lutea. In all the others, i.e., all the ovaries removed during reproductive life, there were hemorrhages of various sizes and due to various causes, normal or abnormal. In this material he studied 264 corpora lutea, 152 before and 112 after menstruation. There was some hemorrhage in all the first group. In 118 it was slight, or as he says, within normal limits. In 34, however, the blood filled more than half the cavity. This, as we have already indicated, should not be taken as an indication of pathological significance. Of the cases after menstruation, in 27 the bleeding was free, while in 85 more than half the cavity was filled. By grouping the cases together, he concludes that 30 per cent of corpora lutea before, and 70 per cent after menstruation showed marked hemorrhage. No effort was made to correlate these findings with the various cyclical phases of corpus luteum development, so that these figures can not be considered a refutation of the opinion we have expressed that it is in the vascularization phase that most hematomas have their origin. This statement, we believe, holds good in spite of Sampson's interesting demonstration of the Muellerian origin of certain ovarian hematomas. As with cysts, the characteristics of the hematoma wall are most important in determining whether or not the corpus is still functionally active.

Corpus luteum of pregnancy (Fig. 8)—The differences between the corpus luteum of menstruation and that of gravidity have been perhaps best described by Marcotty, to whose paper the reader is referred for details. From our present viewpoint it may be emphasized that, generally speaking, the true corpus is larger than the spurious, and that it is much more apt to be cystic. According to Marcotty, this is true more particularly in the first half of pregnancy, the corpus becoming smaller and more solid in the second half. We have had very little opportunity of studying true corpora except those associated with early pregnancy, chiefly of the tubal variety. Fraenkel long ago spoke of the frequency in extra-uterine pregnancy of corpora lutea with large cystic cavities, an observation which we have had abundant opportunity to substantiate. This same statement applies, though perhaps with less frequency, to intra-uterine gestation, for a considerable proportion of the true corpora lutea which we have had occasion to study show cystic lumina filled with clear fluid. Others, again, are almost solid, the cores being made up of well organized connective tissue. Blood is usually not present in the corpus luteum of pregnancy, and even when it is, there seems little doubt of its vestigial nature. In other words, it is not due to recent bleeding, but represents merely the still unabsorbed blood from the stage of vascularization. This view is borne out by the histological picture.

Another important distinction between the two main types of normal corpora lutea—the false and the true—is in the amount of connective tissue laid down along the inner or lumen side of the lutein zone. In the menstrual corpus the fibrous zone is always of moderate thickness, though there are marked individual variations. It merges gradually into the fibrinous network laid down during the vascularization stage. The true corpus, on the other hand, indicates, by its histological structure, its greater stability and permanence. There is a wide layer of fully organized tissue paralleling the lutein zone. In the corpora which are markedly cystic this zone stands out especially sharply as the immediate limiting wall of the cyst cavity.

Pathological Variations in the Corpus Luteum

Corpus luteum abscesses (Fig 9).—The point of follicular rupture—the "stigma"—offers an obvious portal of entry for infection from without. It is not surprising, therefore, that corpus luteum abscesses are very frequent. The first description of the lesion seems to have been by Lange in 1895. Orthmann, in his well known paper, *Die Pathologie des Corpus Luteum,* in 1897, reported 21 corpus luteum abscesses, out of a total of 30 ovarian abscesses which were encountered in Martin's clinic. In other words, corpus luteum abscesses constituted more than two-thirds of all abscesses in the ovaries. Various organisms may be found in the abscesses, as would be expected from the fact that they are usually only incidental to the presence of a general pelvic inflammatory disease. Menge was able to demonstrate gonococci both culturally and by tissue-staining in the abscesses which he studied. Tubercle bacilli have been found by a number of investigators (Orthmann, Hertig, Frank and Gersterberg). Organisms can easily make their way into the corpus luteum lumen from infected tubes, from adjacent abdominal inflammatory disease areas or from the adherent intestines.

There has been some discussion as to whether abscesses develop more frequently in normal corpora or in such corpus luteum derivatives as cysts or hematomas. The general opinion appears to be that the larger abscesses—those which perhaps reach the size of 5 to 16 cm.—are almost always derived from cysts or hematomas, and that the abscesses developing in the normal corpora are always quite small. This point can not be looked upon as settled. Certainly it would seem logical to believe that abscesses might equally well develop from normal corpora, from hematomas, or from cysts. They may be unilateral, or, as in the case of Kehrer, bilateral. This case is of considerable interest in that one of the abscesses presented in its outer wall a wavy, hyaline zone suggesting corpus albicans characteristics.

The wall of the typical corpus luteum abscess is yellowish in color, usually a shade paler than that of the lutein layer of the normal corpus. Its inner surface is

almost always folded and often quite wrinkled, presenting many alveolar markings somewhat similar to those of alligator skin, except that they are much finer meshed. It should at this point be emphasized that many yellow-walled abscesses occur in the ovary which are not of corpus luteum origin. For that matter, it is a well known fact that the walls of many pyosalpinges are of this same yellowish hue. The microscope is usually necessary to make the differentiation possible, and even then it is not always easy.

Under the microscope the lutein layer is obscured by a heavy mantle of inflammatory infiltration, polymorphonuclear leucocytes and lymphocytes both being present in large numbers, their proportion naturally being dependent largely on the stage of the process. Plasma cells may also be found, though usually sparsely. Many new capillaries are to be seen, coursing usually radially toward the lumen. The lutein cells may be detected with greater or less distinctness beneath the infiltrating elements. From the latter they stand out quite sharply as large polygonal cells, arranged in rather tile-like fashion, usually in alveoli. Their nuclei are large and vesicular, the cytoplasm clear and often vacuolated. In many cases their recognition is quite easy; in others difficult and often very doubtful. This is especially true because they are often simulated by large leucocyte-like cells, which may, even in abscesses of other types, lead to the mistaken diagnosis of corpus luteum abscess. These "pseudo-lutein cells," as Kehrer aptly calls them, are not infrequently seen in the walls of pus tubes. Sampson describes them also in the walls of ovarian hematomas, and speaks of their strong resemblance to genuine lutein cells.

Corpus luteum cysts.—The first description of corpus luteum cysts, according to Fraenkel, was given by Rokitansky in 1859. Following this, there was apparently no publication on the subject until the paper of Nagel, in 1887. This author described a number of unilocular cysts which he said were probably of corpus luteum origin, although he did not actually demonstrate this fact except in the case of one cyst. E. Fraenkel, in 1894, describes five cysts which are undoubtedly of corpus luteum origin. The most valuable of the earlier contributions on the subject was probably that of L. Fraenkel, in 1898. This investigator described the histological findings in six corpus luteum cysts, and he uses these findings as a basis for classification, as we have discussed under the next heading. A second paper on the subject by E. Fraenkel appeared in 1899. In 1903 appeared the epoch-making contribution of L. Fraenkel on the function of the corpus luteum. This paper embodied the now familiar Born-Fraenkel theory as to the dominant rôle of the corpus luteum in the physiology of menstruation, and was soon followed by a series of studies by authors, chiefly of the German school, dealing with the histogenesis of the corpus luteum and its relation to menstruation and ovulation. The first of these was by Robert Meyer in 1911, other important studies being reported by Schröder, Ruge, Wallart, Seitz, Miller and Marcotty. These new additions are of great importance in the study of corpus luteum cysts, inasmuch as they have thrown an altogether different light on the discussion of certain relevant problems—the histogenesis of the lutein layer, the time of occurrence and the quantity of bleeding into the corpus luteum, the manner of its retrogression, etc.

Classification of corpus luteum cysts.—The classification of corpus luteum cysts given by L. Fraenkel in 1898 has been the basis for all subsequent schemes of subdivision. This author described six corpus luteum cysts, varying in size from that of a hazel-nut to that of a peach. The contents were yellowish and syrupy, and the lutein wall varied in thickness from 1 to 4 mm. One of these cysts was lined with lutein cells alone. Four showed an inner lining of fibrous tissue within the lutein zone. One, finally, showed an innermost layer of low epithelium, almost like endothelium in appearance. These three varieties have rather generally been referred to in the subsequent literature as Fraenkel's Type I, Type II, and Type III. Only a few of the third type have been described in the literature, among them being at least one (E. Fraenkel), in which the lining epithelium was a single layer of palisade-like cylindrical cells, with round nuclei and well developed cilia. Equally puzzling is the case which Fraenkel says was communicated to him by Orthmann, in which an epithelial lining was said to have been observed directly upon the lutein layer, without the interposition of a fibrous layer, as in Fraenkel's Type III. This case, Fraenkel suggests, may be considered a Type IV. It is of interest to note, however, that all of these epithelium-lined cysts were described before the acquisition of our newer knowledge concerning the origin, histogenesis and life cycle of the corpus luteum, so that considerable doubt is thrown upon the correctness of the interpretation in some of these cases. For example, Fraenkel himself, in discussing Orthmann's case, suggests that the epithelium is probably granulosal in character, if the modern theory of the granulosal origin of the lutein cells is correct.

Although our own material has been quite large, we have encountered no cysts with a definite epithelial lining. We are inclined to believe, on rather circumstantial grounds, that the cysts of this type that have been reported are to be explained in one of several ways. It is possible that some of them may have been, not true corpus luteum cysts, but merely follicular cysts with marked lutein-like transformation of the theca—a not uncommon occurrence, especially with pregnancy. The retention of a layer of granulosa in such a cyst might well produce a rather close simulation of the Type III picture of Fraenkel. Another possibility is that the epithelium in such cases is really the germinal epithe-

lium, which, as is well known, has a strong tendency to flow into cyst cavities communicating with the exterior. As far as Fraenkel's own epithelium-lined cyst is concerned, it is quite possible that the explanation suggested by Cohn is correct. This observer considered that the flat epithelium lining the cyst was of endothelial origin, and he considered the cyst cavity as a large lymphatic space.

Our own corpus luteum cysts, 19 in number, can be divided rather readily into two main groups, corresponding to the first two types described by Fraenkel. There seems little doubt that they represent merely different age phases of the same process, the second type being of course the older stage of development. We have, therefore, designated them, in our own classification, as the recent and the old types of corpus luteum cysts.

Recent corpus luteum cysts (Fig. 10).—There are two distinguishing points of this type of cyst, both indicative of its comparatively recent formation. One of these is the excellent preservation of the lutein cells, and the other is the comparative thinness of the fibrous layer. In some cases the lutein layer is rather thin and ribbon-like, corresponding in development to the vascularization stage of the corpus luteum. The festooning seen in the latter is not noted in the cyst. In others the lutein layer is broader, with perhaps a suggestion of festooning, and with a rather light layer of connective tissue along its inner margin. This picture would suggest that the cyst arose during the stage of maturity of the corpus luteum, or that, if it arose at an earlier stage, a certain degree of development of the lutein layer took place later.

The question which naturally arises at this point is as to whether these cysts are not merely unusually cystic but otherwise normally functioning corpora lutea. We are convinced that this is the case in a large proportion of specimens. Clinically, however, these cases are properly classed under the head of cysts, inasmuch as we are still ignorant of any means of differentiating the normal from the abnormal. For the present, the size of the cysts is perhaps the only criterion which can be suggested. Normal cystic corpora lutea are rarely more than 2 cm. in diameter, and perhaps this size may arbitrarily be selected as the line of demarcation. The largest one of this type of cyst in our collection measured 6 cm., the average size being 4 or 5 cm. The contents of the cyst are usually more or less bloody and of a thick gelatinous consistence. Some cysts, however, are filled with a clear straw-colored fluid.

It is of interest, from the standpoint of etiology, to note that all of these cysts were associated with a pelvic inflammatory disease of one form or another, an observation in keeping with that of Fraenkel (1922), who says that almost every pelvic inflammatory lesion tends sooner or later to the formation of corpus luteum cysts. In 20 of Cohn's 30 cases, also, there was a hyperemic process of one form or another involving the generative organs. In 7 there was tubal pregnancy, in 1 an intrauterine pregnancy, in 5 a chronic pelvic inflammatory disease, in 1 a myoma, in 1 a cervical carcinoma, and in in 5 an acute inflammatory disease leading to luteum cyst abscesses. As an illustration of this provocative effect of surrounding inflammation, he mentions a case in which myomectomy was performed, and at the same time the left ovary removed (and transplanted to the great omentum). The right ovary was perfectly normal. Four and a half months later a second laparotomy was done because of pain and a right-sided mass. Extensive omental adhesions were found, and the right ovary showed a lutein cyst of the size of an orange. It is of interest to note also, that this same association with inflammatory lesions is observed in the case of cattle, according to W. L. Williams, who states that the cause of the extremely common cysts of the corpus luteum in cattle is an infection of the oviduct.

Of especial interest is the study of the menstrual history of these women. In 5 of our 8 cases there was no quantitative disorder of menstruation, either before or after the development of the associated pelvic inflammation. In 1 case, the last period before operation is said to have been one week overdue, and in 2 cases menstruation is noted to have been excessive since the beginning of the inflammatory disease. In no case was there a menstrual syndrome like that in the corpus luteum persistens group (see below) i.e., amenorrhea, followed by prolonged bleeding. Furthermore, the study of the endometrium in 4 of these cases showed no decidua-like hypertrophy, such as is said to be observed in the corpus persistens cases. In all of these cases in which the endometrium was obtained it was found to be of the histological stage of development, which would be expected at the time of its removal, with one exception, unexplainable except on the supposition of a faulty history. The lutein zone of the cyst also corresponded with the menstrual history, so that the assumption appears to be warranted that the lutein cells were still functionally active in spite of the size of the cyst.

Old corpus luteum cysts (Figs. 11 and 12).—The distinguishing characteristics of the older cysts may be inferred by contrast with what has been said concerning the recent cysts. The lutein layer in the extreme cases has so far retrogressed that it is at many points scarcely distinguishable, even though its identity is unquestionable at other parts of the cyst. This fact in itself is proof of the incorrectness of the dictum of L. Fraenkel that lutein tissue must be demonstrated in the entire periphery of a cyst in order to justify its classification as a corpus luteum cyst. In some cysts the lutein cells still form a very sharply defined and well preserved stratum, in others they have, as already stated, very much retrogressed. The fibrous coat also varies much in denseness and in width, these two properties being in a general way in-

dicative of the age of the cyst. In the older specimens the fibrous zone may be considerably wider than the lutein zone.

In a number of these cases the endometrium was also obtained for study. It showed always the histological picture which would be expected from the menstrual history. There was only one apparent exception to this rule, but in this case the history was so contradictory as to be unreliable. Furthermore, in several cases, in addition to the corpus luteum cyst, the ovarian tissue removed was found to contain a normal corpus luteum of the stage of development to be expected from the menstrual history. This we look upon as a very important finding. It suggests at once that these older cysts have apparently dropped out of the physiological cycle altogether, that they no longer exert any effect upon ovulation, and that, in short, they are no longer of any functional value whatsoever. Whether this statement can be extended to all old corpus luteum cysts we can not definitely say, inasmuch as in most of our cases all ovarian tissue had not been removed. Unless both ovaries are available for study, the presence of corpora lutea can not be indisputably excluded.

Eleven specimens of the older type of corpus luteum cyst have been encountered in our material. The average size is somewhat smaller than that of the recent variety, being about 2 to 2.5 cm. The largest measured only 4 cm. The contents are similar in character to those seen in the other variety, being usually brownish or chocolate-colored, though, at times, yellowish or straw-colored. Here again, the association with pelvic inflammatory disease is striking. In all our cases except one there was present a definite salpingitis, and in this one case there was found a large ovarian cystoma with many pelvic adhesions.

The menstrual histories of our patients are also of great interest. Five showed no irregularity whatever; in three there was metrorrhagia; in 1 only a slight irregularity of intervals for the preceding year; in 2 the menstrual histories were not obtainable. In two of the three cases of metrorrhagia there was clinically some suggestion of ectopic pregnancy. In one of these there had been amenorrhea for two months, followed by bleeding for 22 days. On pelvic examination a mass about 6 cm. in diameter was felt in the posterior cul-de-sac. A diagnosis of tubal pregnancy was therefore made. The other patient had had both tubes and one ovary removed at a previous operation for pelvic inflammatory disease. Nine days before her second operation she began to bleed and also to suffer with severe pain in the left side of the pelvis, where a painful mass could be felt. Had the details of the first operation not been known, the logical diagnosis would have been tubal gestation. The operation showed the remaining ovary to contain a corpus luteum cyst of the type we are now discussing, the ovary being firmly adherent to its surroundings.

Such findings bring up the interesting question of distinguishing between tubal pregnancy and certain anomalies of the corpus luteum, especially the one which has been spoken of as *corpus luteum persistens*. Halban, Rubin, and others have called attention to this matter, the usual statement being that it is the corpus luteum cyst which may so closely simulate ectopic pregnancy. Halban reported nine cases of this sort, although he remarks that two of these were complicated by intrauterine pregnancy. Rubin's cases, five in number, were somewhat similar, although in one of them the ovarian lesion is described as a follicle retention cyst and not a corpus luteum cyst.

There can be no question as to the closeness with which tubal pregnancy may be simulated by this ovarian condition, which, like tubal gestation, is characterized by a one-sided mass, together with amenorrhea, followed by persistent uterine bleeding. There is, however, considerable doubt in our minds as to the correctness of the usual interpretation of this curious ovarian abnormality and especially as to whether it is, as Halban and others appear to assume, exactly identical with the condition so often encountered in cattle, and spoken of by veterinarians also as *corpus luteum persistens*. The latter is said to be the most common cause of sterility in cattle, because of the inhibitory influence of the persistent corpus luteum upon ovulation. It is this type of sterility which veterinarians are able to correct by expressing the corpus from the ovary by manual pressure through the animal's rectum. The explanation of this form of sterility in cattle would seem to be clear, if one has the proper conception of the real function of the corpus luteum.

One frequently hears or reads the statement that the latter is the cause of menstruation—or œstrus. This is true only in a properly qualified sense. The corpus luteum is unquestionably the cause of the premenstrual hypertrophic changes in the endometrium, changes whose ostensible purpose is to prepare the endometrium for an impregnated ovum. If pregnancy supervenes, the premenstrual or pregravid endometrial hypertrophy continues to the formation of young decidua. On the other hand, if the ovum which had been given off at the preceding ovulation has not been fertilized, the corpus luteum activity is checked, and the preparation of the endometrium goes for nought. The hypertrophied uterine mucosa involutes, as it were, with the occurrence of the hemorrhage which constitutes the most conspicuous feature of menstruation. Just what the factor is which checks the corpus luteum at a certain point has not as yet been established, but there is good reason to believe that it is associated with the death of the ovum which had been given off at the preceding ovulation, about two weeks before menstruation. It would seem that the extra-follicular life of the unimpregnated ovum is a fairly constant factor and that each ovum, at its dis-

charge from the follicle, possesses a definite potential life span of something like fourteen days. If this factor of ovular influence is disturbed, the rhythm of menstruation is upset. There is much reason to believe, therefore, that the ovum is the dominant factor in the physiology of menstruation.

Theoretically, at least, one of the possible manifestations of ovular abnormality is the so-called corpus luteum persistens. The corpus luteum, instead of ceasing its function at the usual time, persists for a certain period beyond it, so that the menstrual catabolic change in the endometrium is held in abeyance, and the endometrial development may even advance to a pseudo-decidual stage. This would seem to be exemplified in the case recently reported by Curtis, in which a persistent corpus luteum cyst was associated with enormous hypertrophy of the endometrium, which was five or six times as thick as normal.

The problem which presents itself in these cases, as they occur in the human female, is to exclude pregnancy, which is simulated so perfectly ("Scheingravidität"). How can we be sure that the amenorrhea and the ultra-menstrual endometrial hypertrophy are not, after all, due to the presence of an impregnated ovum in the generative tract? So far as we know, the only efforts to settle this point are the few mentioned by Meyer. In two cases this investigator, working with Weishaupt, made serial sections of the entire uterus and tubes, and was unable to find the ovum. He mentions one similar study carried out by Runge and alludes also to still another by Rokitansky. The latter, however, assumed that pregnancy had occurred, even though he was unable to find the ovum.

All of the 3 cases of corpus luteum persistens reported by Meyer were associated with carcinoma of the cervix. In one case menstruation was absent for 32 days, in the other two for 33 days. The corpus luteum in these cases presented a striking picture. The lutein layer was from 2 to 4 mm. thick. There was no hemorrhage into the cavity, which was sometimes, though not always, markedly cystic, the corpus being perhaps as large as a cherry. The lutein cells showed no retrogressive characteristics, and especial importance was placed upon the absence of fatty change, as in the corpus luteum of pregnancy. Meyer states that in two cases examined from this standpoint the theca changes so often seen in pregnancy were absent. He lays down as the essential points in the diagnosis of corpus luteum persistens, therefore, the occurrence of amenorrhea, the finding of a mature cystic or non-cystic corpus luteum without evidence of retrogression and fatty change, and the functional ultra-menstrual hypertrophy of the endometrium. The latter resembles young decidua, and must of course be distinguished from such pathological conditions as hyperplasia of the endometrium.

Our skepticism of this usually accepted theory concerning cases of the so-called corpus luteum persistens type was first aroused by the observation noted above, that in the real corpus luteum cysts, either recent or old, mimicry of tubal gestation was not, as a rule, observed. Our suspicions were further aroused by an instructive case which was encountered in our study, and which is worth repeating in brief.

CASE OF INTRAUTERINE PREGNANCY WITH SPONTANEOUS INCOMPLETE ABORTION WITH CYSTIC CORPUS LUTEUM OF PREGNANCY, SIMULATING ECTOPIC PREGNANCY

L. T. (w) Gyn. No. 22128. Gyn. path. Nos. 22166 and 22189. Admitted May 13, 1916.

P.H. Appendectomy for acute appendicitis in 1908. *Menstrual:* Onset at 12. Reg. q. 4 weeks. Duration 4 d. with variable amount of pain. Moderate flow. No intermenstrual bleeding before P.I.

L.M.P. Mar. 16, 1916. Preceding Feb. 15, 1916. *Marital:* Married 6 yrs. 2 children—aged 5½ and 3 yrs. No miscarriages.

P.I. Onset Apr. 30 (6 wks. after last period) with dull pain in left lower quadrant, only severe on walking. Since onset of P.I. she has had slight vaginal bleeding. On May 12, after examination in dispensary, she had a severe attack of bleeding which awoke her. Entered hospital next morning and was taken immediately to operating room.

Pelvic examination: Outlet marital. Moderate relaxation. Bloody vaginal discharge. Cervix small, firm, and lacerated. Fundus twice the normal size, a little soft; good position. Small cystic mass, size of hen's egg, in left fornix. Tenderness in both fornices. Ovary on right normal, freely movable. Left ovary not distinguishable from mass. W.B.C. 9,500. Hb. 92%.

Diag.: 1. Extrauterine pregnancy, left: (2) Possibly cystic ovary with retained membranes.

Operation: May 13, 1916. (1) Enucleation of cyst of left ovary. No blood found in abdominal cavity. Tubes normal. (2) Dilatation and curettage.

Post-operative course: uneventful.

Discharged: May 28, 1918.

Pathological report: Retained membranes. Cystic corpus luteum of pregnancy.

Here, then, was a case very strongly suggesting ectopic pregnancy, and in which a corpus luteum cyst was found instead. Fortunately, however, this patient was curetted at the same time, and the microscopic examination of the curettings gave definite evidence of a recent pregnancy.

We are strongly inclined to the belief that many other of the cases reported in the literature as instances of corpus luteum persistens are really cases of very early death of the impregnated ovum, and that the corpus luteum cysts described as having been found in such cases are probably nothing more than cystic corpora of pregnancy. Suggestive in this connection is the casual statement of Halban that 2 of his 9 cases of corpus luteum persistens were complicated by pregnancy.

It is quite probable that a microscopic examination of the uterine mucosa might prove negative, even though there had been an abortive impregnation, so to speak. The exceedingly early stage of the process, together

with the possibility, as brought out in the recent investigations of Evans, that complete embryonic resorption may occur, makes it almost impossible, it seems to us, to exclude pregnancy in any such cases. Practically the same idea has been expressed by Rubin, who suggests that the retention cysts in the ovary may result secondarily from early embryonal deaths in either the uterus or the tube.

Of course, there is the theoretical possibility that a corpus luteum may be rendered functionally persistent by some ovular anomaly independent of pregnancy, and that the syndrome above described may thus be produced. In view, however, of the scientific fact that this same syndrome is not infrequently found where there is a demonstrable pregnancy, the burden of proof would seem to lie on those who would explain these cases on the non-pregnancy basis.

Corpus luteum hematomas.—There can be very little doubt that, as a rule, these are due to excessive hemorrhage into the lumen during the stage of vascularization. It is during this stage that bleeding takes place as a normal phenomenon, although commonly it is moderate in amount, the blood forming a narrow zone within the lutein layer. Not infrequently, however, the entire lumen is filled, and at times the bleeding is so abundant that a hematoma of considerable size may result. As in the case of cysts, it is not always easy to draw the line between the normal and the abnormal. A similar basis for classification, into recent and old hematomas, would appear to be logical, and this plan we have followed.

Recent corpus luteum hematomas (Figs. 13 and 14.—Under this heading we have included those specimens in which the lutein layer was well preserved and in which the connective-tissue deposit was slight in amount or almost absent. Many of these corpora lutea, we have no doubt, are still functionally active, although clinically they are properly spoken of as hematomas. Some investigators believe that there is also some hemorrhage into the lumen at or just before the menstrual periods. In the study of a large number of mature corpora, however, we have seen no evidence of such menstrual hemorrhage, an observation which agrees with that of most authorities. The well marked fibrous layer so characteristic of this stage would make improbable the occurrence of any hemorrhage into the lumen at this time.

Ten of our hematomas may be placed in this group. They are comparatively small, averaging about 2 cm. All except one were associated with pelvic inflammatory disease. There was nothing of very striking significance to be gleaned from the menstrual histories. In 2 menstruation was perfectly normal, in 2 it had always been somewhat irregular but otherwise normal, while in 5 metrorrhagia had been noted since the onset of the pelvic disease. In one case only the patient is said to have gone beyond her period about 10 days. In view of the coexistent pelvic inflammatory disease in this group of cases, it is rather difficult to attach much importance to the frequent occurrence of metrorrhagia as a symptom. In the few cases in which the endometrium was obtained for study, it was found to correspond in a general way with the stage of the menstrual cycle represented by the lutein zone of the hematoma, thus suggesting the persistence of active function in the latter.

Old corpus luteum hematomas (Fig. 15).—In these the lutein zone has undergone marked retrogressive changes, especially atrophy and fibrosis, so that it is often impossible to distinguish it as a definite layer. In the light of what has been said in the discussion of the preceding group, it apears likely that the corpus is blighted in the vascularization stage by the inundation of blood, and that there is an immediate onset of retrogressive changes. It is of interest to note that the hyalinization so characteristic of the normally retrogressing corpus luteum (corpus albicans) is not observed in most of these hematomas, indicating that probably they have not passed through the cycle of changes of the normal corpus. In a small proportion, it is true, hyaline and fatty changes are seen, suggesting that in these there has been no interference with functional activity.

In cases in which the lutein stratum is poorly marked, it is difficult to distinguish the hematoma from the ordinary follicular variety, for in the latter there may be at times a lutein-like transformation of the theca interna, making it morphologically not unlike a genuine lutein layer.

In certain cases, indeed, it is impossible to make this distinction between a hypertrophied theca layer in a follicular hematoma and a retrogressive lutein layer in a corpus luteum hematoma. Many of the older authors spoke of lutein hematomas as arising either from follicles or corpora lutea, although this view is scarcely allowable if we concede the correctness of the modern idea concerning the histogenesis of the lutein cells. At times light is thrown upon the question by other factors, such as the rather sharper demarcation from the surrounding ovarian stroma which is observed with corpus luteum structures, a point emphasized by Cohn. Finally, the difficulty is not made easier by the fact that the lutein strip is very uneven in its distribution and its development. As with cysts, it is not unfrequently absent at some points and well developed in others, thus again contradicting the old dictum of Fraenkel as to the presence of the lutein layer throughout the circumference of cysts, and presumably also of hematomas.

The connective-tissue layer within the lutein zone varies much in thickness and density. In the old hematoma it may be several times as broad as the lutein zone. In the less advanced cases, on the other hand, it is of course much narrower. Its inner margin is rarely clear cut, the fibroblastic process pushing into the blood irregularly along the entire edge.

Our material contains 23 specimens which we have placed in this category. All except two were associated with pelvic inflammatory disease, and in one of these two a myoma was present. The size varies between 1.5 cm. and 2.5 cm. Six patients were regular in their menstruation both before and after the development of the pelvic disease; two had always been irregular, and the remaining 15 had been normal before, but abnormal after the onset of the pelvic trouble. This abnormality was in the nature of either menorrhagia or metrorrhagia, the two being about equally divided. Here, as with the recent hematomas, a rather large proportion show menstrual excess, although here again we have the overshadowing importance of the associated inflammatory disease as a conflicting factor. This clinical finding is certainly worthy of consideration, although we are as yet unable to offer any satisfactory explanation for it.

Corpus albicans cysts (Fig. 16).—In the study of our material we have encountered a few cases of definite cystic change in corpora albicantia. This condition is far less common than is the ordinary corpus luteum cyst. An excellent study of this type of cyst was published by Koebner in 1908, although a number of authors had previously reported such cases. Pfannenstiel, in Veit's Handbuch, described corpus luteum cysts with wavy, hyaline walls, which evidently belong to this class. He believed that such cysts represent a later stage of the ordinary corpus luteum cyst, that the latter, in other words, goes through the same series of retrogressive changes in its walls as does the corpus luteum itself. Others who described corpus albicans cysts are Bulius, Nagel, Bollenhagen, and Steffeck. The last named author's case is of especial interest because of the large size of the cyst, which was as big as a man's head. It had two cavities, each lined with a dense wavy hyaline tissue.

The three cases reported by Diepgen are also of importance because in two of them there was a lutein layer which gradually merged into hyaline tissue. This Diepgen considered to be evidence of the correctness of Pfannenstiel's theory. Incidentally, it may be pointed out that cysts of this variety, which surely belong in the corpus luteum category, do not conform to the requirement of L. Fraenkel, that the lutein layer must be demonstrable throughout the entire circumference. As has already been shown, this requirement would rule out many cysts which are undoubtedly of corpus luteum origin, and hence it should not be considered as of any great value for purposes of classification.

Corpus albicans cysts are practically always of small size, rarely measuring more than 1 to 1.5 cm. in diameter. There are occasional exceptions to this rule, the most extreme being Steffeck's case, which has already been mentioned. The walls are very dense, and of chalky-white color. They are made up, microscopically, of tissue exactly similar to that seen in the normal corpus albicans, the most conspicuous element being, of course, the broad, festooned, amorphous, acid-staining hyaline layer which replaces the original lutein zone. This layer may itself form the inner margin of the cyst wall, although more frequently there is a light layer of organized tissue obviously derived from the core of the corpus albicans. The cases which are most difficult to explain are the few which are said to have shown a definite epithelial layer within the fibrous tissue. Various theories have been put forward as to the origin of this epithelium, as in the case of the epithelium-lined corpus luteum cyst. Some believe that the epithelium is derived from the germinal epithelium, some that it is tubal in origin, and still others, including Koebner, consider that it is to be looked upon as endothelial in character, the cyst cavity representing a large lymph space, as Fraenkel suggested for the corresponding type of corpus luteum cysts. This view, however, would hardly explain those cases in which the epithelium is of a definitely cylindrical character. There are some who believe that the epithelium represents an upper layer of unchanged granulosa cells, only the lowermost layers of the latter undergoing transformation into lutein cells. The advocates of the von Baer theory of the theca origin of the lutein cells would, no doubt, explain the epithelium as corresponding to the granulosa. Neither of these views is very plausible. Nor are we impressed with Koebner's explanation that the epithelium in some of these cysts may be due to hemorrhage under the basal membrane at the time of follicular rupture, the lifted layer being capable of independent vitality, while the remainder of the granulosa presumably goes on to lutein transformation. In our own material we have not encountered any corpus albicans cysts which show an epithelial lining.

With regard to the origin of these cysts, the evidence seems to be in favor of the correctness of Pfannenstiel's view that they are end-products of corpus luteum cysts. Indeed, L. Fraenkel appears to be almost the only author who considers that they arise from cystic softening of the already formed corpus albicans. Assuming that they do arise from corpus luteum cysts, the question suggests itself as to whether all corpus luteum cysts terminate as corpus albicans cysts, just as normal corpora lutea eventuate in corpora albicantia. Against this view is the very great disparity in the incidence of the two types, the corpus luteum cyst being very common, the corpus albicans variety relatively rare. Furthermore, the latter are practically always very small, the former often quite large. So that it would seem that only the small corpus luteum cysts could terminate as corpus albicans cysts.

Our own view is that the corpus albicans cyst in almost all cases is merely the end result of the normal cystic corpus luteum which we have already described, and that it is not to be looked upon as possessing any pathological significance. The only exception to this would seem to be in the case of such exceptionally large cysts of this variety as that reported by Steffeck. One

is almost inclined to doubt that this cyst was of the corpus albicans type, for apparently this assumption was based only on the demonstration of a hyaline layer in the wall. The presence of two cavities would, it seems to us, also speak against Steffeck's theory of the origin of the cyst.

There are several points of interest in the 5 specimens of corpus albicans cyst which are included in our series. In the first place, as a rule, they are considerably smaller than the corpus luteum cysts proper, their average size not being over 1 cm. They are included among the cysts, in spite of their small size, because of their special nature and their relative infrequency. They might perhaps just as well be called cystic corpora albicantia. The small size of these structures would suggest also that they are probably the ultimate products of normal cystic corpora lutea rather than of large corpus luteum cysts. This would be further suggested by the fact that in 3 of our 5 cases there was no pelvic inflammatory disease, which is almost constant with other corpus luteum cysts. In one of these 3, there was a carcinoma of the cervix; in another a simple follicular cyst of the ovary; in the third there was no demonstrable pelvic pathology. In 2 cases there was inflammatory disease, probably merely coincidental. It is also interesting to note that 4 of the 5 cases were in women between 41 and 48 years of age, although the significance of this is not clear. In 4 cases menstruation was more or less increased and irregular. In at least 2 of these this abnormality was easily explained by the presence of a carcinoma in one and a polyp in the other. We do not believe that these small hyalinized corpora can exert any influence upon the menstrual function.

Just as we have found two types of both cysts and hematomas, so we have found two corresponding types of corpus albicans cyst. In the one the wall of the cyst is formed by the wavy eosin-staining hyalinized lutein zone, which often retains, at least, a suggestion of its characteristic wavy contour. In these there is little or no connective-tissue layer. In another group there is seen a well marked inner zone of fibrous tissue, this forming the immediate lining of the cyst.

Corpus albicans hematomas (Fig. 17).—Of this type, which we have not found described in the literature, we have three specimens. All are quite small, the largest measuring 1.3 cm. All were associated with inflammatory disease in the pelvis, although this is perhaps of no significance. Two of the 3 histories showed menorrhagia, although, as we stated in connection with the cysts, there is no reason to believe that these obviously retrogressive structures exert any influence on menstruation. Histologically, they are quite similar to the albicans cysts, with the exception that the contents are hemorrhagic rather than clear. This blood is probably a persistence of the physiological hemorrhage of the vascularization stage, as it does not seem possible for hemorrhage to occur in the retrogressive stage, with its avascular hyalinized lutein zone.

Combined types.—While we have, for the sake of clarity, aimed to make the classification of these conditions as sharp as possible, it must be remembered that specimens are encountered which it is difficult to place definitely under any one of these heads. Combination forms are not infrequent, especially in the case of cysts and hematomas. There is no sharp dividing line between these, for cysts often show a moderate amount of hemorrhage, though usually of the zonal type. We have been guided mainly by the degree of hemorrhage into the cavity, classifying as hematomas those specimens wherein the hemorrhage was the striking factor, filling the lumen and often causing great distention. In a similar manner there are transition forms between hematomas and abscesses, i. e., hematomas with a partially purulent content and hematomas whose walls show marked evidence of infection.

Corpus luteum neoplasms—Luteomata (Glynn).—From time to time there have been reports in the literature of ovarian tumors, which for one reason or another were believed to have been of lutein origin. In a recent paper on the subject Glynn has collected 14 such cases from the literature. He accepts all of these as of lutein origin. He quotes Turnbull as giving the following main differential characters of lutein bodies, viz: "(1) the stellate core of fibrin or granulation tissue, (2) the absence of clearly defined acini, and (3) the alternation of centrifugal and centripetal trabeculæ." Glynn presents evidence indicating that the cases which have been reported in the literature as large ovarian hypernephromata are probably of lutein rather than of suprarenal origin. For example, with the exception of one doubtful case, there is no recorded instance of the finding of accessory suprarenal tissue in the ovary. He speaks also of certain histological differences between the two types of tumor, and emphasizes the fact that whereas suprarenal hypernephroma produces striking changes in sex characters, these changes were absent in the recorded cases of ovarian hypernephromata. For such reasons as this, he believes that the latter were really of lutein origin.

To this discussion we can add very little from our own investigation. We believe there can be little doubt as to the lutein origin of certain tumors, more especially of such clinically unimportant growths as the one reported in 1919 by Ewing. This growth measured 2.5 × 3.5 cm. and microscopically resembled an enlarged corpus luteum. We have also observed in certain malignant tumors of the ovary that in some areas the cells are morphologically very suggestive of lutein cells. We have, however, thought this evidence much too flimsy to warrant this conclusion, especially since a complete study of such specimens has usually shown in other parts a

structure which stamps the tumor quite definitely, usually as carcinoma.

We do not believe that the evidence for the lutein origin of all of the 14 cases collected by Glynn is at all convincing. This statement, we feel, applies to all of his "Sub-group Two," embracing "5 briefly recorded cases." Several among the 9 cases which he appears to accept as indubitable, we are also strongly inclined to believe, are open to serious question. We feel sure, for example, that his Case 2, reported by Schaller and Pförringer, in 1899, as an instance of carcinomatous degeneration following cystic transformation of the corpora, is merely an example of multiple theca-lutein cyst of the ovary associated with hydatid mole. In this case such a mole was expelled from the uterus twelve days after the operation. Furthermore, the description corresponds exactly with that of the many similar tumors which have been found in association with hydatidiform mole or chorioepithelioma, as will be mentioned below.

The case of Grouzdew (1903) also appears to be quite questionable, especially since the tumor, after operation, recurred in the lymphatic glands and on the peritoneum as a spindle-celled sarcoma. Similar criticism might be made of several other cases, so that, all in all, the evidence for the lutein origin of certain ovarian tumors is as yet rather inconclusive. Theoretically, there is of course, no reason why such neoplasms may not occur, and they almost certainly do. They must be quite rare, however, or else we have not learned to recognize them as yet. For the present not much will be gained by theoretical speculation on the subject.

Allied Conditions

Multiple lutein cysts of the ovary (Figs. 18, 19).—This interesting condition does not, strictly speaking, have a place in the discussion of diseases of the corpus luteum, inasmuch as, from the standpoint of origin, it appears to be in no way connected with the latter. It is so closely allied to the corpus luteum, however, and it has in the past been so closely associated with it in the minds of many investigators, that at least a brief mention of the subject would seem to be necessary to make this discussion complete. The condition has been rather fully treated in a recent paper by one of us (Novak) to which the reader is referred for details.

Multiple lutein cysts of the ovary are found in association with a certain proportion of hydatidiform moles or chorioepitheliomata. The first adequate description was by Stoeckel in 1902, and since then Seitz, Wallart and others have published studies of the subject. Without going into details, we may summarize by saying that the evidence is almost conclusive that the lutein cells lining the cysts in cases of this type are of the type of theca-lutein cells rather than granulosa-lutein cells, as in the corpus luteum.

During pregnancy there is normally a greater or less degree of hypertrophy of the theca cells of atretic follicles, giving them a somewhat lutein-like appearance (theca-lutein cells). The stimulus for this transformation emanates presumably from the embryo, probably from the trophoblast. To quote from our previous paper, "with hydatidiform mole or chorio-epithelioma, it would seem that the trophoblastic overgrowth is responsible for an enormous increase and perhaps perversion of this causative stimulus, so that the ovarian response is correspondingly increased. The number of atretic follicles is increased and the lutein-like changes in the theca cells become pronounced. Even the theca cells of follicles, which have long since become atretic or which have already become converted into corpora fibrosa, are wakened into activity by this stimulus, to which they appear to be specifically sensitive. This explains why the theca lutein cells are so often found beneath a deep layer of connective tissue, and why they often appear to be buried deep in the ovarian stroma." It was this characteristic, no doubt, which led Schaller and Pförringer to interpret the condition as "a carcinomatous infiltration," as we have discussed above. Such a picture as that presented in Fig. 19 settles absolutely, to our mind, that these peculiar lutein cells are really of theca rather than granulosa origin, for they are here seen forming an imperfect layer beneath the intact granulosa.

Finally, it may be added that these multiple lutein cysts are evidently entirely secondary to the intrauterine condition, i. e., the hydatidiform mole or the chorioepithelioma. This is attested by the fact that they have a strong tendency to spontaneous disappearance as soon as the intrauterine condition is removed.

Summary

A *sine qua non* in the study of the pathological anatomy of the corpus luteum is a knowledge of the normal life cycle of this structure—its histogenesis, its cyclical variations, and its manner of retrogression. The corpus luteum exhibits individual variations within physiological limits. The most important of these are cystic distention of the corpus and an excessive degree of hemorrhage into the lumen during the stage of vascularization. The most important pathological conditions affecting the corpus luteum are (1) abscess formation (2) cyst (3) hematoma (4) neoplasm. Corpus luteum abscesses are very frequent, comprising a considerable proportion of all ovarian abscesses. It is probable that they arise from infection of normal corpora lutea, corpus luteum cysts, or corpus luteum hematomas. In regard to cysts and hematomas, it is often difficult to draw a line between these and the individual variations observed in the corpus luteum under normal conditions, as mentioned above. The size of the structure and the histological character of its limiting wall are the two most important factors in this differentiation. Both

cysts and hematomas are usually associated with pelvic inflammatory disease. Corpus luteum cysts may be subdivided into (1) the recent type, in which the lutein layer is well preserved and in which there is little or no fibrous tissue deposit between it and the contents, and (2) the old type, in which the age is indicated by retrogression of the lutein zone and by the heavy, organized layer on its lumen side.

Analyses of the menstrual histories of recent corpus luteum cysts indicate that, in general, the lutein zone corresponds to the developmental stage which would be expected at the time, and that the structure, in spite of its cystic nature, is still of functional importance. The older cysts, on the other hand, have entirely dropped out of the physiological cycle, and exert no influence on the menstrual function.

These findings throw doubt upon the prevalent impression that corpus luteum cysts have a tendency to delay menstruation, and that they bring about a clinical syndrome easily mistakable for that of tubal pregnancy (amenorrhea followed by prolonged bleeding, together with a one-sided mass). While it is possible that there may exist a definite endocrinopathic or other entity of this so-called corpus luteum persistens type, it is exceedingly difficult in cases of this kind, to rule out the possibility of a very early abortion of a tubal or uterine pregnancy. The possibility of such an error is illustrated by the case we have reported in this paper. The difficulty of eliminating pregnancy is enhanced by the fact that complete resorption of the embryo may conceivably occur, as has been shown by Evans and others.

Corpus luteum hematomas are commonly due to excessive hemorrhage into the lumen during the stage of vascularization. They permit of the same subdivision into recent and old as was suggested in the case of the cysts. Furthermore, these types bear the same relation to the menstrual phenomenon as the corresponding types of cysts.

Cysts and hematomas are at times encountered whose walls present the characteristic corpus albicans structure. With the exception of the few very large cysts of this character which have been reported, but which are of doubtful nature, the corpus albicans cysts and hematomas are probably merely the end-results of the normal cystic or hemorrhagic corpora lutea which we have described.

Various authors have described ovarian tumors of supposedly lutein origin. There is no reason why such tumors should not occur, and we have observed growths, chiefly carcinomas, in which morphologically such an origin was suggested. Some at least of the 14 cases of lutein cell tumors collected from the literature by Glynn are of a very doubtful nature, and further investigation will be necessary before the possibility of a lutein origin of certain ovarian tumors can be accepted as definitely established.

The condition spoken of as multiple lutein cysts of the ovary, found with some cases of hydatidiform mole and chorioepithelioma and sometimes erroneously designated as multiple corpus luteum cysts, is due to a widespread lutein-like transformation of the theca cells in the atretic follicles, which are greatly increased in size and number.

In conclusion, we wish to express our appreciation to Dr. Thomas S. Cullen for placing at our disposal the wealth of material in his laboratory and to Mr. Herman Schapiro for the excellent photomicrographs which he made for us.

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Fig. 1.

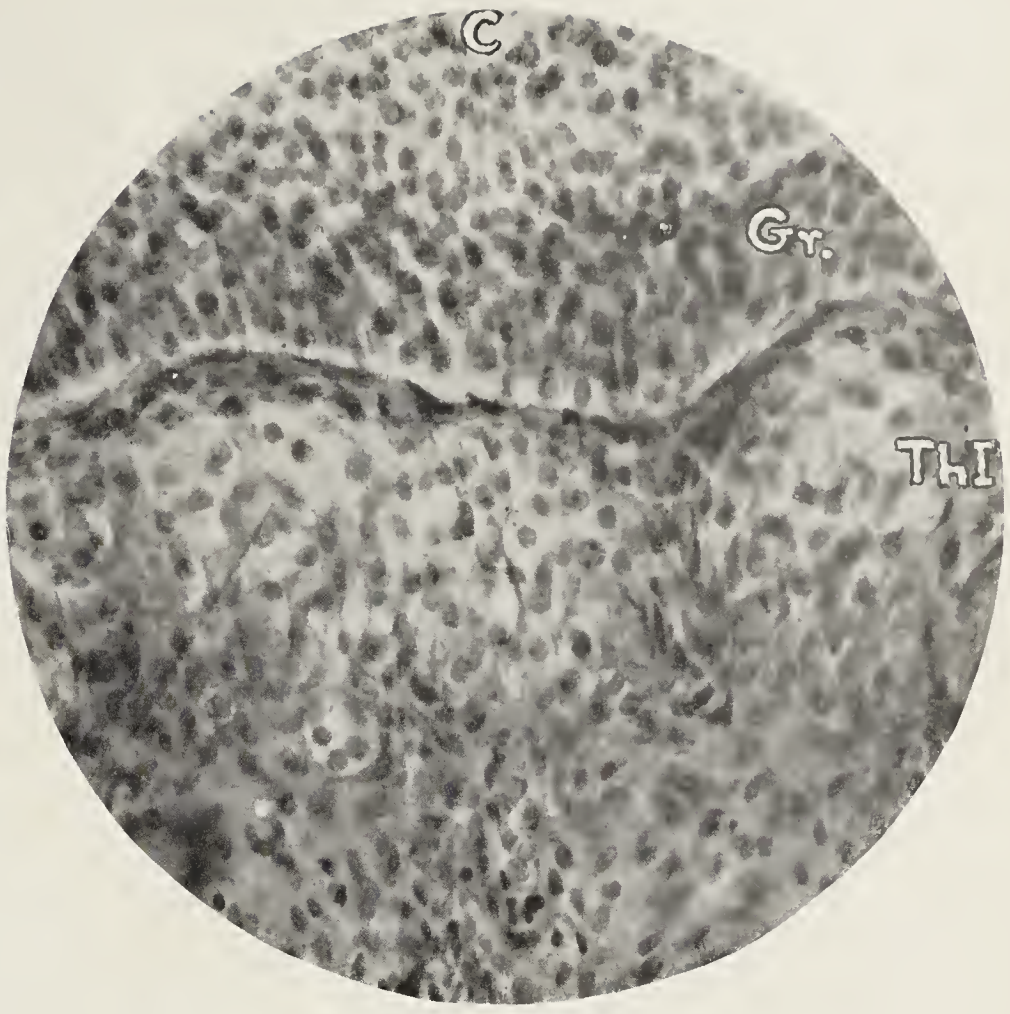
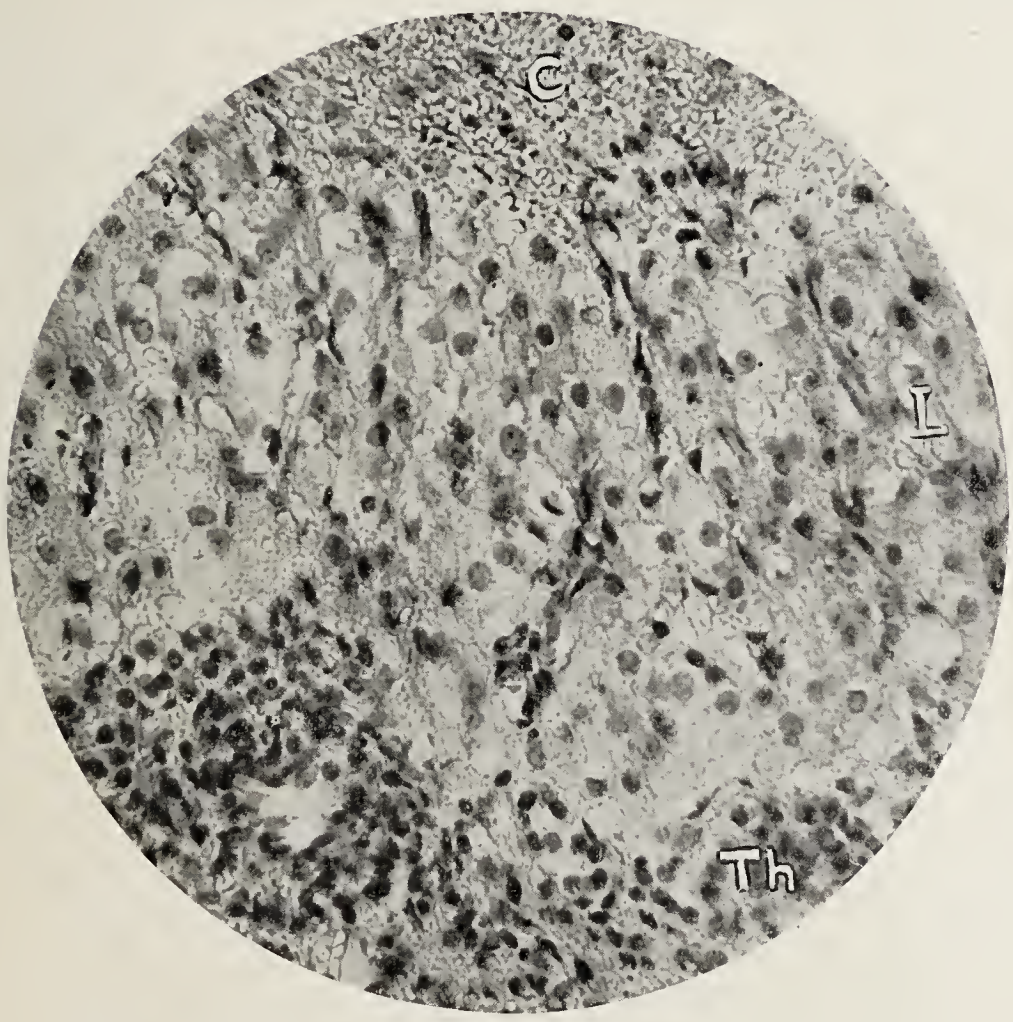


Fig. 2



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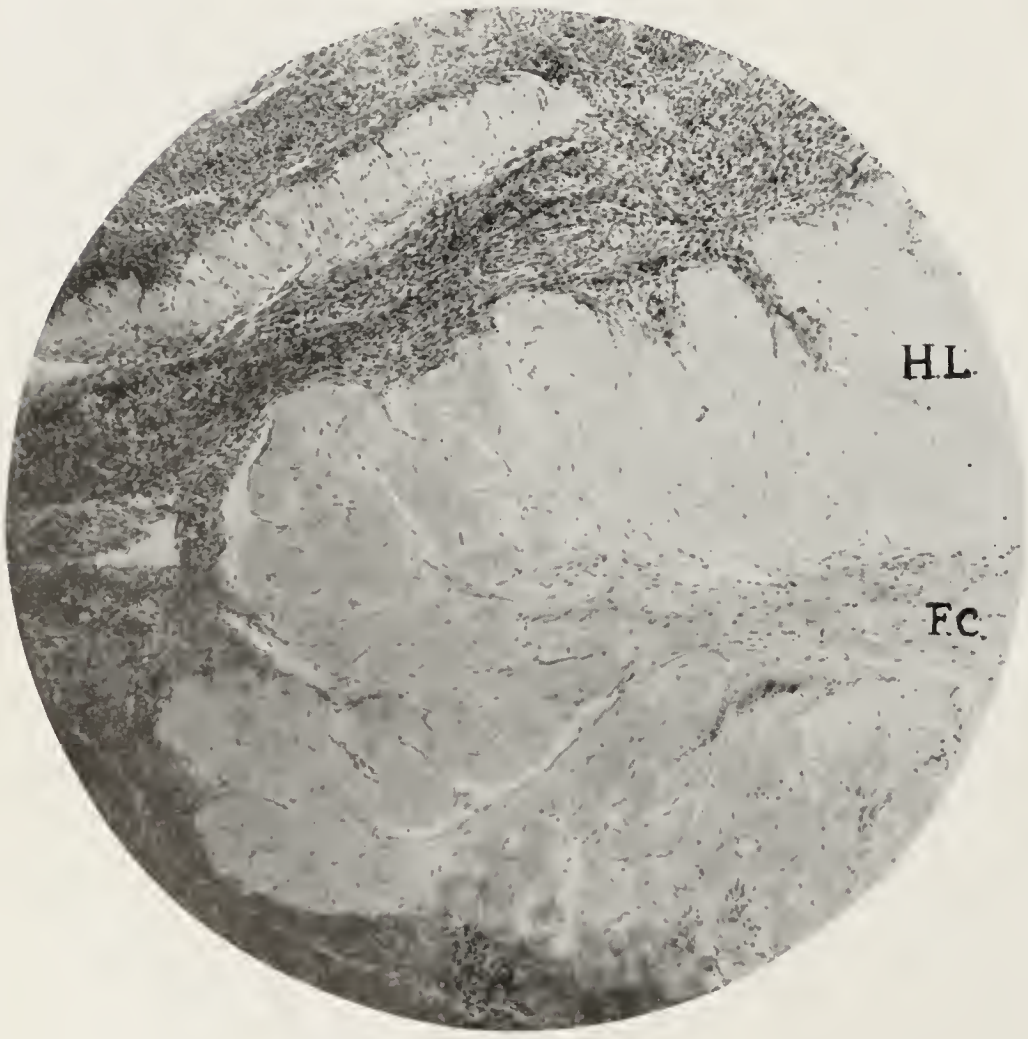

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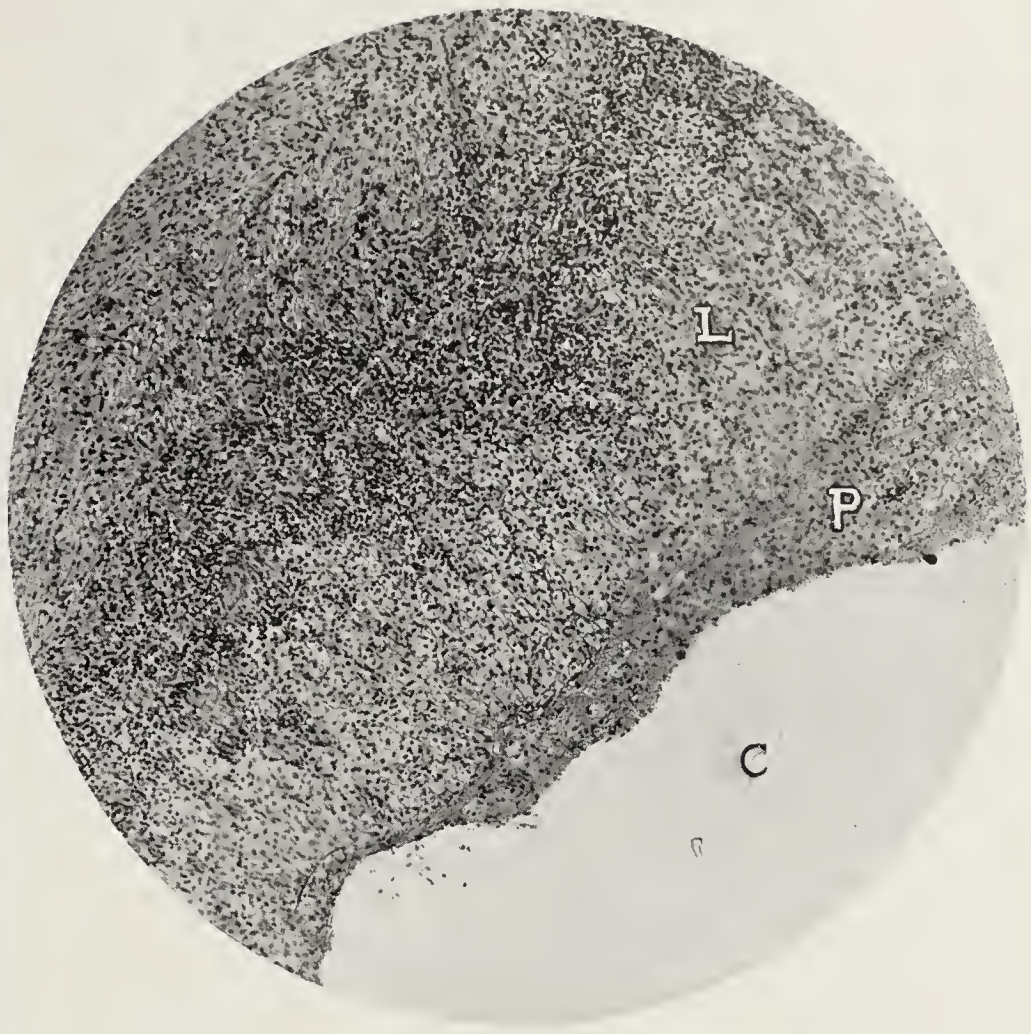

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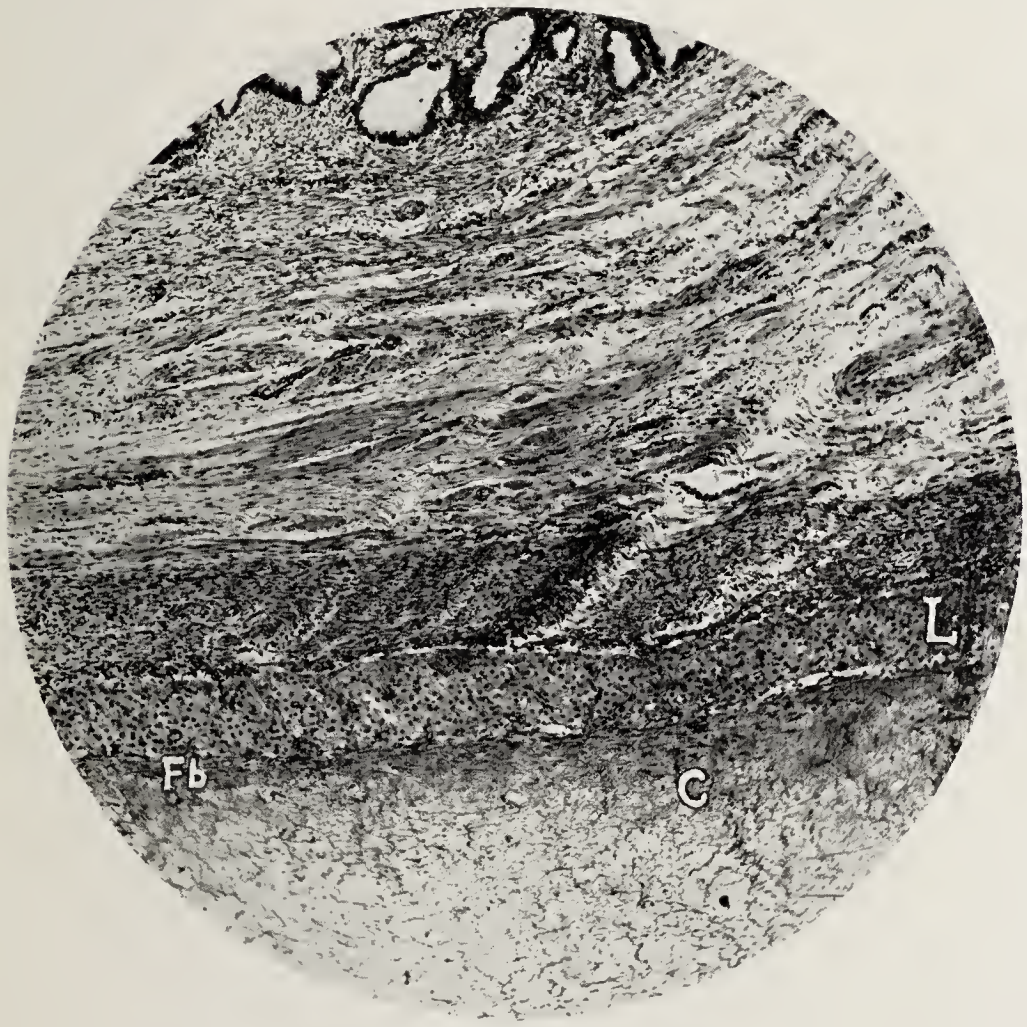

Fig. 10.

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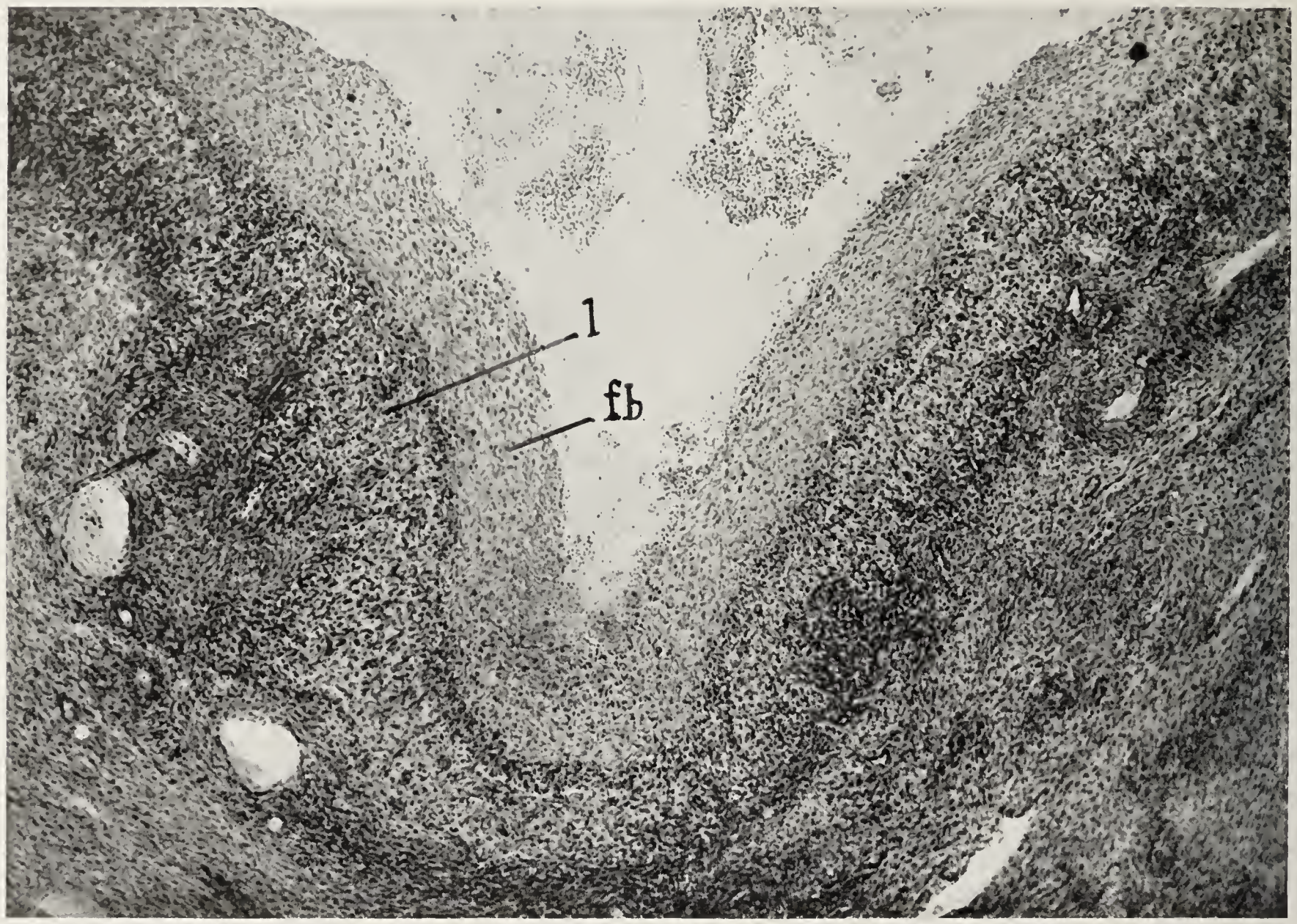

Fig. 12.

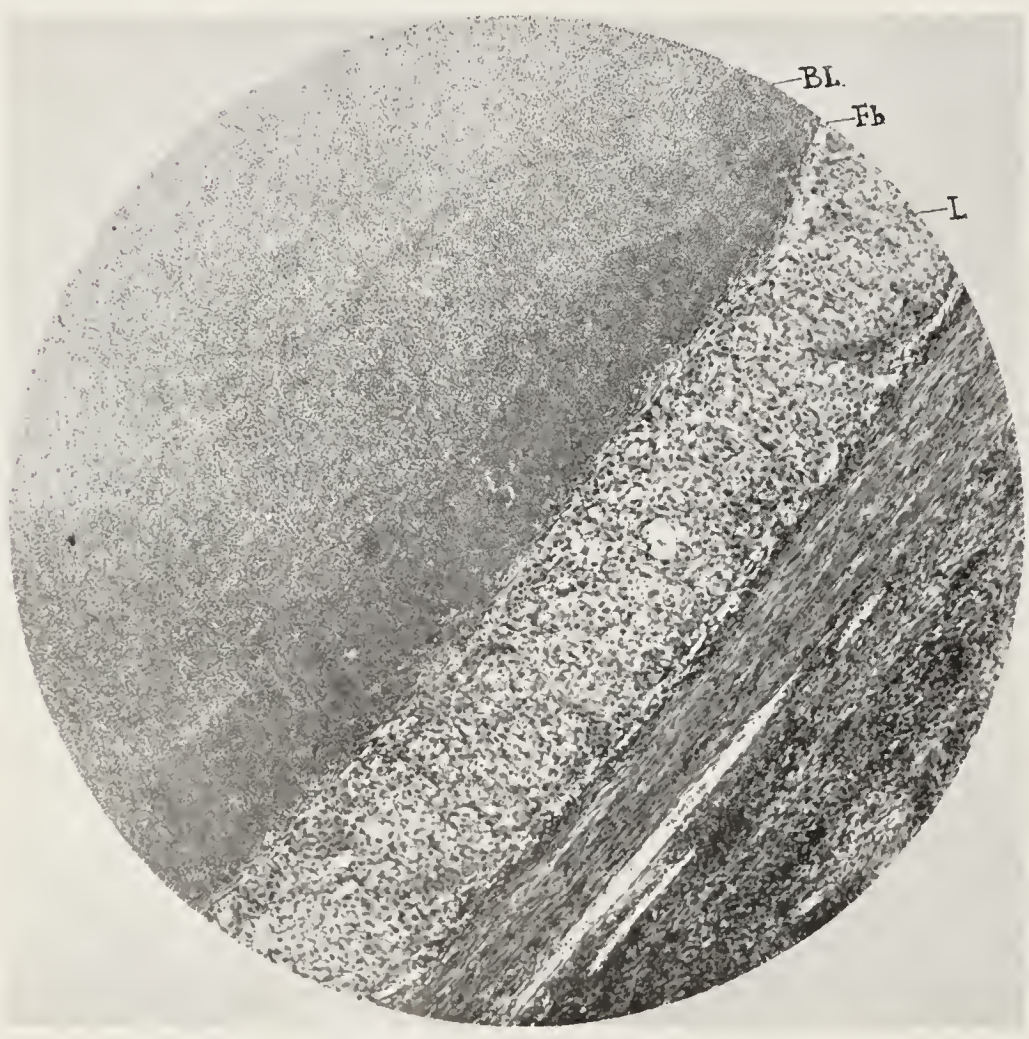

Fig. 13

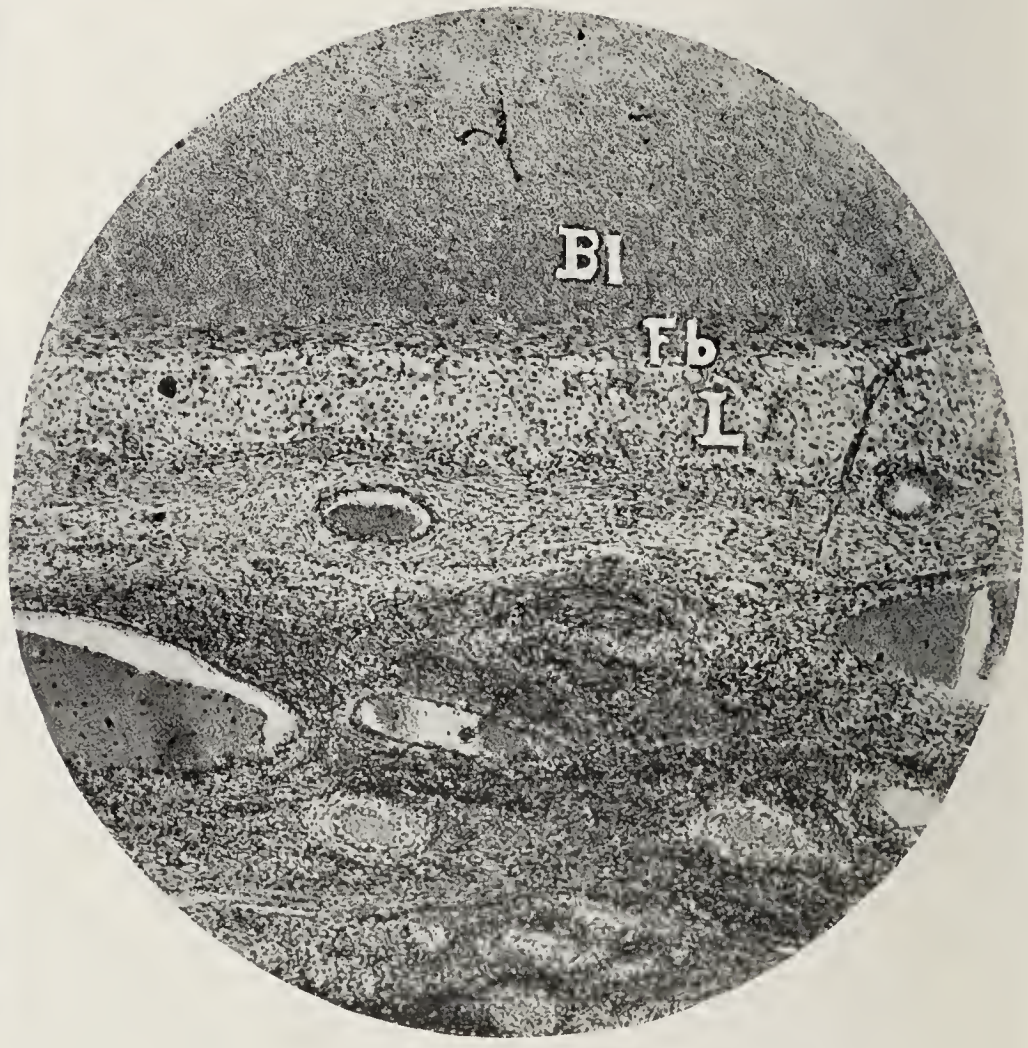

Fig. 14.

Fig. 15

Fig. 16.

Fig. 17.

Fig. 18.

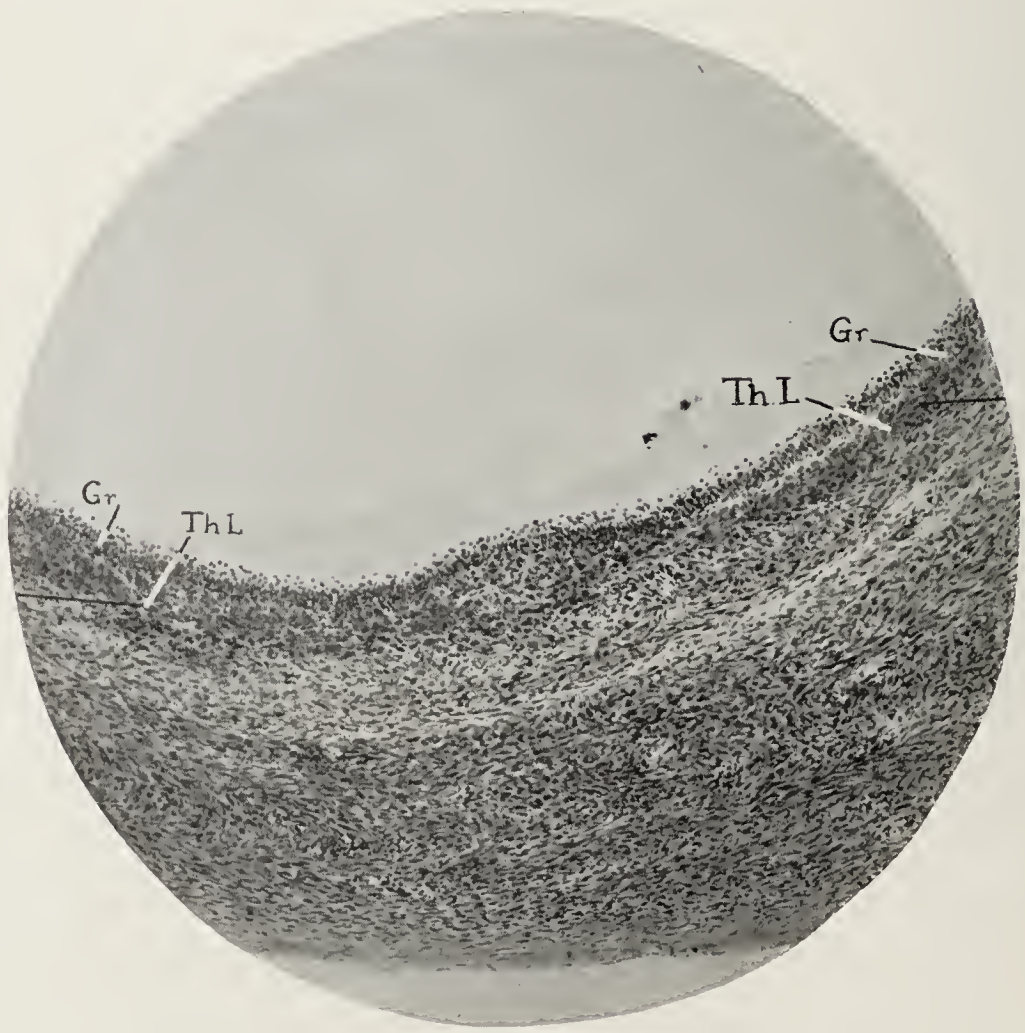

Fig. 19.

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Idem: Ueber Frühstadien abortirformen der Corpus luteum Bildung. Arch. f. Gynäk., 1914, CIII, 544.

DESCRIPTIONS OF PHOTOMICROGRAPHS

Fig. 1.—Wall of an early corpus luteum, removed on tenth day of cycle, showing changes characteristic of the stage of proliferation (low power). (Gr.) membrana granulosa. (Th. I.) theca interna.

Fig. 2.—High power of corpus luteum shown in Fig. 1. (c) cavity. (Gr.) membrana granulosa. (Th. I.) theca interna. Note at this stage the theca interna cells resemble lutein cells even more than the granulosa cells.

Fig. 3.—Wall of corpus luteum in stage of early vascularization; sixteenth day. (High power.) Blood vessels from the theca are invading the granulosa layer (1) which now possesses definite lutein characteristics while the theca cells (th.) have lost to a certain degree their former lutein characteristics. We note in this stage for the first time a zone of hemorrhage into the cavity.

Fig. 4.—Wall of mature corpus luteum, showing typical thick convoluted layer of large lutein cells (1) and a definite fibroblastic layer (fb) lining the central cavity (c). The picture does not extend peripherally far enough to show the so-called paralutein cells.

Fig. 5.—Corpus luteum showing beginning retrogression. Note the marked invasion of the retrogressing lutein cells by fibrous tissue, which also forms the central core.

Fig. 6.—A corpus albicans. Note the completely hyalinized old lutein zone retaining its characteristic festooned outline.

Fig. 7.—Mature hemorrhagic corpus luteum (first day of menstrual cycle), the cavity of which is filled and distended somewhat beyond the normal average with blood. The hemorrhage has not distended the corpus greatly, it being slightly less than 2 cm. in diameter and the festooned arrangement of the lutein layer being still preserved. The blood does not appear fresh, probably having been extravasated during the period of vascularization.

Fig. 8.—Cystic corpus luteum of early pregnancy. Bordering on the lutein layer is a well developed fibrous layer (fb) much better developed than in the average spurious corpus. Among the fibres a few r. b. c. are found, fewer than in the average corpus unassociated with pregnancy.

Fig. 9.—Wall of corpus luteum abscess. In the portion of the wall shown, the lutein layer (L) is quite well preserved but heavily infiltrated with polymorphonuclear leucocytes and round cells. A portion of the sanguineo-purulent content (P) is clinging to the lutein wall.

Fig. 10.—Wall of a recent corpus luteum cyst. This cyst measured 5 cm. in diameter and had a thin coffee-colored content. Note the well preserved lutein layer (L) with only a few fibroblasts (fb) along its inner border. These two facts indicate recent formation of the cyst.

Fig. 11.—Wall of an older corpus luteum cyst. Diameter only 1.5 cm. Note the lutein layer (L) showing signs of retrogression and the very heavy fibrous layer within it. These two facts indicate that the cyst is not of very recent formation.

Fig. 12.—Wall of a still older corpus luteum cyst. The lutein layer (L) shows signs of retrogression and is partially replaced by fibrous tissue (fb) invasion. The innermost fibrous layer is extremely heavy.

Fig. 13.—Wall of a recent corpus luteum hematoma. This hematoma measured 2.5 cm. in diameter. The festoooned arrangement of the lutein layer is no longer present, due to distention by blood. The lutein layer (L) shows no retrogressive changes, the blood in the cavity is very well preserved, and there are only a few fibroblasts (fb) lining the lutein layer. All of these facts point to the recent formation of the hematoma.

Fig. 14.—Wall of a recent corpus luteum hematoma. Lutein layer (L) still well preserved, but the better development of the fibrous lining (fb) indicates that it is not of as recent origin as the hematoma shown in Fig. 13.

Fig. 15.—Wall of an older corpus luteum hematoma. Note that the lutein layer (L) has been invaded by fibrous tissue and a very heavy fibrous layer (fb) has been deposited around the periphery of the enclosed blood-clot.

Fig. 16.—Corpus albicans cyst. Note the completely hyalinized old lutein layer (H. L.)

Fig. 17.—Wall of corpus albicans hematoma. Note the completely hyalinized lutein zone (H L) and the hyalinized fibrous zone (H F) immediately surrounding the blood-filled cavity (Bl.)

Fig. 18.—Showing lutein-like cells in "multiple lutein cysts" associated with hydatidiform mole.

Fig. 19.—Section from cyst wall of tumor shown in Fig. 18. Note the definite though retrogressive granulosa layer (Gr), beneath which is a zone of dark-staining polygonal theca lutein cells (Th. L.) Morphologically, these cells are similar to those shown more clearly in Fig. 18.

LIPEMIA RETINALIS

By W. S. McCann, M. D.

(From the Chemical Division of the Medical Clinic, Johns Hopkins Hospital)

Very recently Gray and Root [1] have reported two cases of lipemia retinalis and have collected 26 others from the literature. Though only 28 cases have been reported so far, the experience in this clinic would indicate that the condition is much more common than one would suppose from the infrequency of case reports. It has been observed twice in this clinic during the last 18 months from among 150 patients with diabetes. One of these cases is reported here because certain observations have been made which throw new light on the explanation of the appearance of the retina in certain cases of lipemia.

Case Abstract

Charles G. (Med. No. 49195) a schoolboy, aged 15, was readmitted to the medical service on March 13, 1923, on account of diabetes mellitus, for which he had been previously admitted in April, 1922. A complete report of his case on the first admission has been given in a previous publication.[2]

The essential points were as follows:

Family History.—Paternal grandmother probably had diabetes.

Past History.—Scarlet fever at 7 years, measles at 9 years of age. Typhoid fever immediately preceding the onset of diabetic symptoms, which began in October, 1921.

Present Illness.—From October, 1921, until his admission to this hospital in April, 1922, continual efforts had been made to make him gain weight by overfeeding. When he was admitted first he was in a state of severe acidosis, which slowly cleared up under diet regulation. He left the hospital on June 1, 1922, with a high carbohydrate tolerance. He continued sugar-free for four months on diets furnishing 50 grams of protein, 110 grams of fat, and 50 grams of carbohydrate. After this he occasionally passed sugar in his urine. When this occurred, the patient had been able to become sugar-free by taking nothing for one day except 1000 grams of green vegetables. Glycosuria, however, began to be noted with increasing frequency. The patient began to lose weight, to suffer from obstinate constipation and frequently with severe attacks of vomiting. On account of these symptoms he was advised to return to the hospital for further observation. He left his home in Florida on March 11, the urine being sugar-free. When he arrived at the hospital on March 13, he was in a serious condition. The positive findings on admission were as follows: *Temperature 99°F. Pulse 96. Respirations 20.* The patient was markedly under weight. He lay quietly in bed, without evidence of drowsiness or hyperpnoea. The breath had the odor of acetone. Mentally he was alert and cooperative.

There was marked pyorrhoea about all the teeth. The gums were spongy and bled easily on pressure.

Eyes.—The pupils were equal in size, regular in outline and reacted promptly to light and accommodation. All extraocular movements were normally performed. The ocular tension was normal. Visual acuity 10/10. The fields normal on rough tests. Media clear.

On examination of the fundi one was struck by the appearance of white vessels against a salmon-pink background. An attempt was made to reproduce this appearance in the drawing in Figure 1. This was only partially successful; there were many fine details which could not be brought out.

The abnormalities of the fundus were all confined to the retinal vessels. The larger trunks, both arteries and veins, appeared red within the optic cup. As the vessels crossed the optic disc, the red color became more and more confined to the center of the vessel and there was a shading through moss rose to grey at the sides of the vessel wall. At a short distance from the disc all red color disappeared from the vessels, which were of a striking whiteness. Arteries and veins looked very much alike; the light streak was lost, giving an appearance of flatness to the vessels. The white color extended into the finest visible vascular radicles, indeed it seemed to make small vessels visible which could not ordinarily be seen. This whiteness of the fine vessels of the optic disc itself gave it an appearance of pallor. The color of the retina itself was essentially normal. The choroidal markings were distinct.

The *thorax* was long and narrow. Respiratory movements were normal. Lungs: vocal fremitus was normally transmitted and normal resonance was obtained on percussion. On the initial examination a few fine râles at the apices were recorded, but this finding was not confirmed and roentgenograms of the chest revealed no abnormalities.

Cardio-vascular system.—The heart was normal. The vessels of the arm seemed somewhat hard for a boy of 15.

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[1] Gray, Horace, and Root, H. F.: Lipemia Retinalis. J. Am. Med. Assn., **80**: 995, April, 1923.

[2] McCann, W. S., Hannon, R. R., Perlzweig, W. A., and Tompkins, E. H.: Studies of diabetes mellitus. II. The results of treatment by diet regulation with reference to basal requirement and the ketogenic antiketogenic balance. Arch. Int. Med., July, 1923.

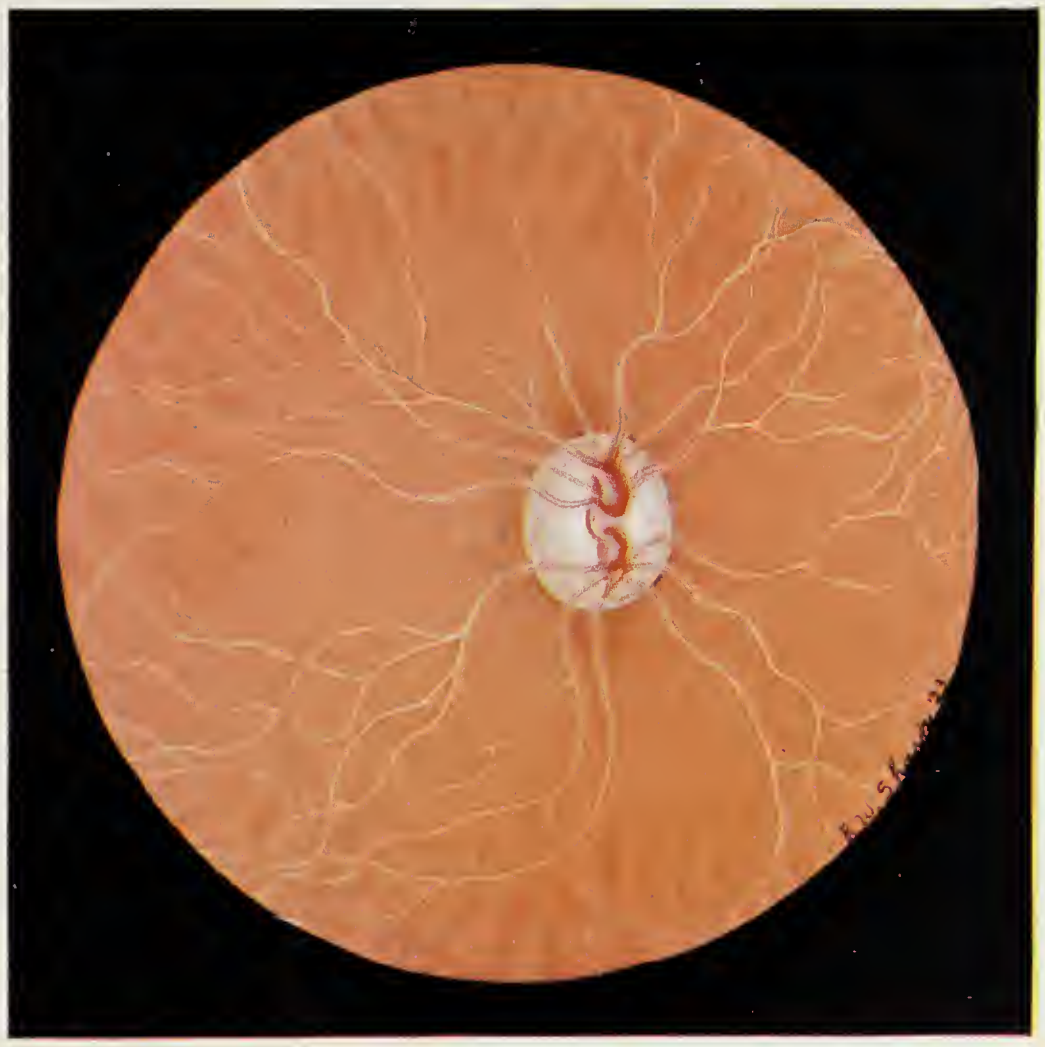


Fig. 1.—Appearance of retina on March 15th.

The capillary vessels of the base of the nails of the fourth and fifth fingers of the right hand were examined with a microscope by Miss E. B. Carrier, who gave the following description:

"Lombard technique. The capillary bed and underlying venous plexus are clearly seen. The shape of the capillary loops is normal, the arterial limb being narrower than the venous. The only condition not previously observed normally is an occasional very fine direct anastomosis by means of a fine branch connecting the arterial and venous limbs. The color of the loops is the normal red, the background clear white.

"The stream in the capillaries is very slow and is interrupted by frequent white spaces about the size of a leucocyte. These are more numerous than leucocytes normally seen passing through the capillaries. They may be fat or plasma spaces. One can count 23 or 24 passing through a loop per minute, although the stream is extremely slow so that it takes a corpuscle 3 or 4 seconds to pass around a loop. It is a continuously steady stream and does not show periods of stasis."

No other abnormalities were observed on physical examination except that the tendon reflexes were obtained with difficulty, the patellar reflexes requiring reinforcement.

Laboratory findings, Blood examination.—R.B.C. 4,010,000, W.B.C. *7,600*; hæmoglobin 100%. The differential count showed 47%polymorphonuclear leucocytes, 47% lymphocytes, and 6% large mononnclears and transitionals. Fat droplets seen of various sizes up to 20 micra. These take Sudan III.

Blood drawn from a vein was deep red in color, but on standing showed a cream line. The plasma after centrifugalization was milky white.

The CO_2 combining power was only 16.2 vols. per cent. The blood sugar was 0.253%.

Urine.—clear, yellow, specific gravity 1032, reaction acid, sugar ++, albumin O, acetone and diacetic acid ++++. Microscopic examination negative.

The progress of the patient under treatment is shown graphically in Figure 2, in which are recorded all data regarding blood and urine analyses, diets, and an estimation of the quantities of food-stuffs actually metabolized per diem. For calculation of the food-stuffs metabolized the following data are used.

Daily urine N. gms.×6.25=protein grams (P).

Carbohydrate intake—urine sugar=carbohydrate utilized (C).

Basal requirement of calories=basal metabolism+ 10%during period of strict confinement to bed.

$$\frac{\text{Basal requirement}-4.1\ (P+C)}{9.3} = \text{Fat grams metabolized.}$$

In referring to the record of Figure 2, it will be seen that the acidosis had been brought well under control by dietary measures alone before insulin was given. The lipemia retinalis continued until March 20th when it began to disappear. It was noted to have disappeared completely on March 22nd. The remarkable thing is that on March 22nd the percentage of total fat in the blood determined by Bloor's method [3] was actually higher than it was on March 15th. The following are the figures:

	Total blood fat gms. per 100 c.c.	Cholesterol mgms. per 100 c.c.
March 15	9.5	865
March 22	10.8	940

These analyses were made by Mr. Caspar Folkoff.

In spite of the higher figures for fat on analysis, the plasma of March 22nd was not as milky on gross examination as it was on March 15th. This corresponds with observations made by Wishart [4] in a recent article on blood fats, in which she states that samples of plasma containing a marked excess of fat may be clear, and that the gross appearance of fatty plasma does not always correspond with the analytical results.

The graphical record of the case shows the degree of improvement of the patient as regards blood sugar, alkaline reserve, and carbohydrate tolerance.

He was discharged with a diet furnishing 2000 calories, requiring a total of 30 units of insulin daily. He has continued this treatment at home, has gained in weight and strength and feels normal in every way.

For further details of the methods of diet regulation and use of insulin in this clinic the reader is referred to previous publications.[2,5]

Discussion

It is a matter of great interest to determine just how the appearance of lipemia retinalis is produced. From their own studies and from a review of the literature Gray and Root [1] believe it to be due to an excess of fat in the blood. The threshold for the appearance of retinal changes they believe to lie between 3-6% of blood lipoids determined by Bloor's methods.

It is clear, however, that the total percentage of fat in the blood can not be the sole factor in the production of the abnormal appearance of the retinal vessels. In the case just described the total fat content of the blood was slightly higher after the vessels had resumed their normal appearance. The appearance of the plasma itself had changed, however, as it was much more turbid and milky on the 15th of March, when retinal changes were well marked, than it was on the 22nd after the retina had become normal-looking. This would indicate that the

[3] Bloor, W. R.: Studies on Blood Fat. II. Fat absorption and the Blood Lipoids. J. Biol. Chem., 1915, XXIII, 317.

[4] Wishart, Mary B.: Lipemia. I. Analyses of blood lipoids in diabetic animals and patients. Jour. Metab. Research, II, 199, August 1922.

[5] McCann, W. S., and Hannon, R. R., and Dodd, K.: Studies of Diabetes Mellitus. III. The use of the pancreatic extract insulin in the treatment of diabetes mellitus. Bull. Johns Hopkins Hosp., July, 1923.

state of aggregation of the fats or lipoids has more to do with the appearance of the retina than the total lipoid content.

Whenever the laboratory reported turbid or milky plasmas it has been customary to make at once a special ophthalmoscopic examination. Lipemia sufficiently marked to be grossly visible is fairly common with diabetic conditions and yet lipemia retinalis was detected only twice in about 150 of such patients, each of whom had one or more ophthalmoscopic examinations.

On March 19, 1923 an analysis of the total blood fat of another diabetic patient, who was in the same ward as Charles G., showed 8.9% fat and 0.780% cholesterol. A special examination of the eyes of this patient failed to reveal a lipemia retinalis.

In spite of the high content of fat the gross appearance of blood freshly drawn from a vein is not abnormal. The blood was distinctly red. It is very difficult to see how the circulation of red blood through a small vessel should give it a white appearance. In this connection, the direct observation of the capillaries of the nail-bed is important in that it showed that the capillaries were normally red. It leads one to the conclusion that the whiteness of the vessels is due either to a change in the vessel wall or to a change in the content of the perivascular lymph spaces.

In an effort to see whether a change had occurred in the walls of arterioles and venules a piece of skin and subcutaneous tissue was removed and examined by Dr. W. G. MacCallum, who reported no abnormalities in the structure of the vessel walls. In view of these findings it is believed that the abnormal appearance of the retinal vessels may be due to a film of fatty lymph in the perivascular spaces or to fat deposits in the adventitia. It was impossible to carry the investigation to the point of histological examination of the retina. Of the two patients in whom the condition was noted in this clinic, the first died a few hours after admission; an autopsy was performed, but permission to examine the eyes was refused.

Summary

The case of a patient with diabetes mellitus with lipemia and lipemia retinalis is described. Evidence is given to show that the appearance of the retinal vessels is not dependent on the total fat content of the blood, for the lipemia persisted after the retina had resumed its normal appearance. Moreover, a case was observed in which the total blood fat percentage was 8.9 without any change in the appearance of the retina.

Direct observation of the capillaries of the nail-bed showed them to be normal in appearance. The shed blood was red. No change in the structure of small skin vessels was observed on histological study. A tentative explanation of the white appearance of the retinal vessels is offered, namely, that the vessel may be surrounded by fat containing lymph or fat deposited in the adventitia of the vessels so that light is abnormally reflected or refracted, and that the appearance might depend on the physical state of the fats or lipoids of the blood rather than on their total quantity.

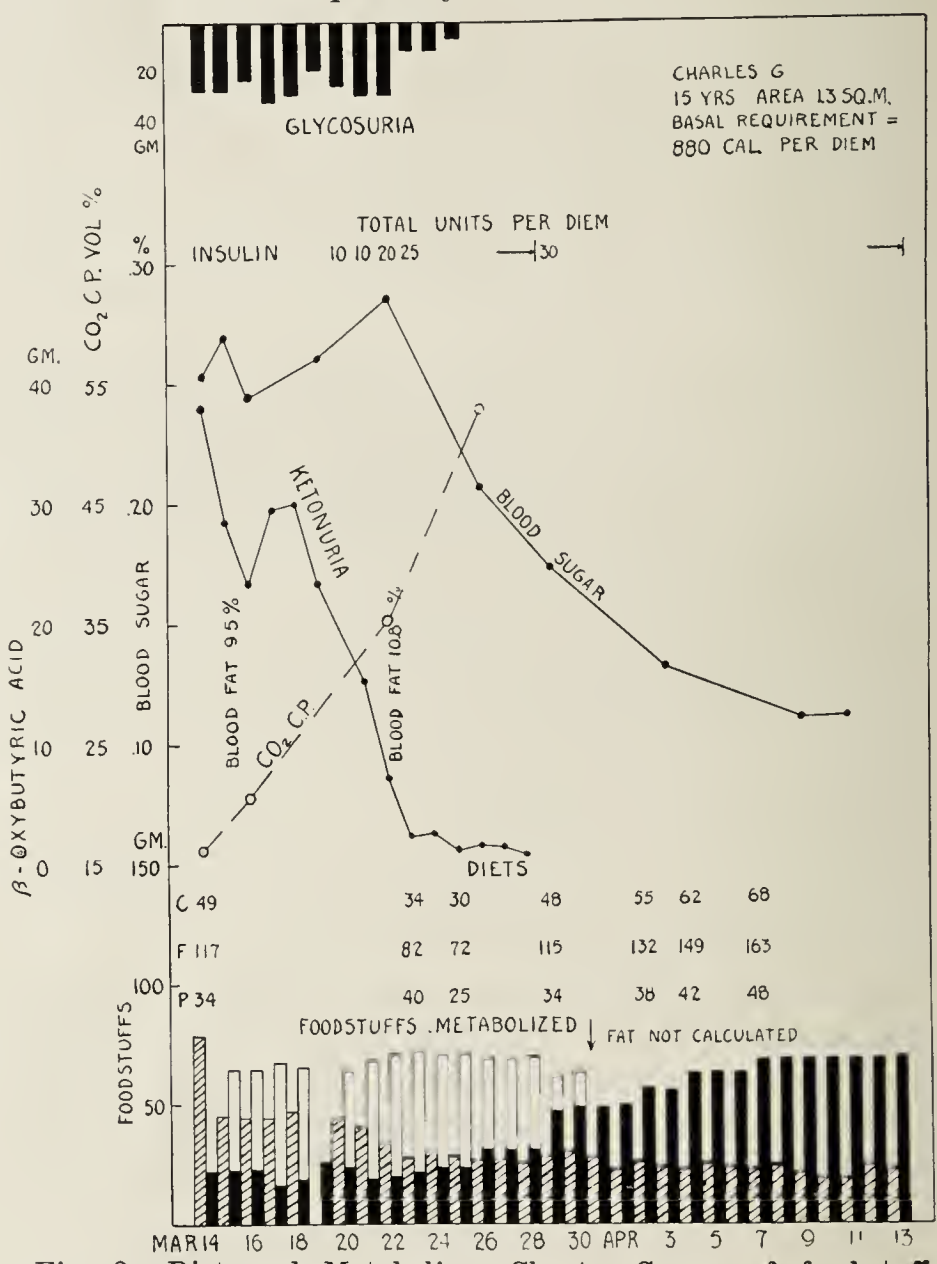


Fig. 2.—Diet and Metabolism Chart. Grams of food-stuffs metabolized daily are shown in columns at bottom as follows: protein—cross hatched, carbohydrate—solid black, fat—unshaded. After March 31 the fat metabolized was not calculated, as the patient was out of bed.

Thanks are due to Miss Elizabeth Sharp for her kindness in furnishing the illustration.

THE GAS EXCHANGE IN DISEASES OF THE THYROID GLAND

By John T. King, Jr.

(From the Medical Clinic of the Johns Hopkins Hospital.)

The almost universal use of the measurement of oxygen absorption as an equivalent of the measurement of the basal heat production, or the basal "metabolic rate," is based upon two widely accepted theories of gas exchange. The "Tissot" method, which depends chiefly for its accuracy upon that of the oxygen determination, has its basis upon these same two principles.

The first of these is the theory of *caloric equivalents* of oxygen and carbon-dioxide, respectively, and is derived from the table of caloric values of Zuntz and

Schumburg, to be found in a modified form in Lusk's Science of Nutrition.[1] If we examine this table, we find that the caloric value of a liter of oxygen is affected very little by the change of the respiratory quotient $\left(\frac{\text{Vol. of } CO_2 \text{ eliminated}}{\text{Vol. of } O_2 \text{ absorbed}}\right)$, whereas there is a considerable discrepancy in the caloric values of carbon-dioxide as the respiratory quotient moves from 0.707 to 1.00. This may be illustrated graphically in the following figure.

Diagram illustrating the fluctuation of the caloric equivalents of oxygen and of carbon-dioxide, respectively, with change in the theoretical respiratory quotient.

R. Q.	Caloric Equivalent of Oxygen	Caloric Equivalent of Carbon-Dioxide
0.707	—3.7%	+13.5%
			
1.00	+3.7%	—13.5%

It is seen at a glance that, whatever the respiratory quotient, the heat value (equivalent) of a given amount of oxygen will be altered only 7.4%, while the theoretical heat equivalent of carbon-dioxide may fluctuate 27.0%.

This fact—for we accept it as a fact—has naturally been very influential in causing most clinical workers to use oxygen absorption rather than carbon-dioxide elimination as an index to the heat production.

However, this whole consideration has presupposed: (1) that alterations in the relation of CO_2 elimination to oxygen absorption, as measured over short periods of time from the respiration, are really indications of a change in the actual relation of these two gases in the body's metabolism, and hence indications of qualitative changes in the metabolism of food-stuffs; and (2) that oxygen absorption is as accurate an index to the heat production in disease as it is in health. It has not been sufficiently borne in mind, as will be shown later, that changes in the "respiratory quotient" may be merely indications of temporary alterations in the gas exchange through the lungs and may not be true indices to the theoretic, or actual, respiratory quotient of the body tissues for the period of time over which the gas exchange has been measured. In diabetes, carbon-dioxide should not be used as an index to heat production on account of the lowering of the true R. Q. in this disease. This objection cannot be applied *a priori,* however, to any other condition, provided the subject is "basal," for, as MacLeod[2] points out, "In numerous other diseases investigated by DuBois and others no qualitative change in the combustion processes in man has been brought to light."

The second chief consideration that has caused O_2 absorption to be used as an index to heat production is the phenomenon of the "washing out" of CO_2, which follows superventilation of the lungs. We are all familiar with the fact that carbon-dioxide can be readily washed out of the blood by overbreathing, while the oxygen absorption is little, if at all, affected.[3,4]

These two theories—the theory of the relative constancy of the caloric equivalent of oxygen and the relative inconstancy of the caloric value of carbon-dioxide, and the theory of the "washing out" of carbon-dioxide by superventilation—have created an argument in favor of oxygen as an index to heat production that has seemed perfectly conclusive. It has not been considered necessary to carry the theories into practice and to establish by experiment to what extent they are applicable to gas exchange work and to what extent they may be superseded by other influences.

The analysis in this paper will have to do with the theory of caloric equivalents, and the effects of superventilation will be considered in a following report.

In a previous contribution[5] an analysis of a number of one-hour periods of chamber calorimeter observations showed that carbon-dioxide elimination was a better index to heat production, as measured by the chamber calorimeter, than was oxygen absorption. The results were as follows:

	Average Error in Correlation	
	CO_2 and Direct Calories	O_2 and Direct Calories
Benedict and Carpenter..	4.93%	8.39%
Soderstrom, Meyer and DuBois.....	4.73%	5.70%

Palmer[6] has criticized this report to the effect that the above figures of caloric equivalents were obtained almost entirely from normal subjects. This same criticism may be applied to the use of oxygen as an index to the heat production in disease. The highly valuable reports of Benedict and his associates from the Carnegie Institution, worked out from normal subjects, have been

[1] Lusk, G.: Science of Nutrition. W. B. Saunders Co. Phila., 1919.

[2] Physiology and Biochemistry in Modern Medicine. C. V. Mosby Co. St. Louis, 1919.

[3] Benedict and Carpenter: Food Ingestion and Energy Transformation. Pub. of the Carnegie Inst., 1918.

[4] Hendry, Carpenter and Emmes: Boston Med. and Surg. Jour., 1919, CLXXXI, 368.

[5] King, J. T., Jr.: Johns Hopkins Hosp. Bull., 1921, XXXII, 277.

[6] Palmer, W. W.: Nelson's Medicine. New York and Edinburgh, 1920.

NAME	Body Surf. Linear	CO_2 per hr. (gms.)	CO_2 per sq. meter per hr. (gms.)	O_2 per hr. (gms.)	O_2 per sq. meter per hr. (gms.)	Cals. per hr. direct	Cals. per sq. m. per hr.	Dev. of CO_2 from avg. (gms.)	Dev. of O_2 from avg. (gms.)	Dev. of cals. from avg. (cals.)	REMARKS
Max W.	1.72	36.19	21.04	34.79	20.22	103.52	60.2	+2.28	+2.83	+3.5	Basal, Restless
		39.28	22.84	37.60	21.86	125.28	72.8	+4.08	+4.47	+16.1	Basal, Restless
Max W.	1.73	32.77	18.94	31.90	18.44	87.44	50.5	+0.18	+1.05	—6.2	Basal, Quiet
		37.19	21.49	34.38	19.87	123.60	71.4	+2.73	+2.48	+14.7	
Max W.	1.70	35.58	20.93	33.13	19.49	105.53	62.1	+2.17	+2.10	+5.4	Basal, Restless
		35.94	21.14	34.25	20.15	113.14	66.5	+2.38	+2.76	+9.8	Basal, Restless
Max W.	1.70	31.79	18.70	30.79	18.11	93.60	55.1	—0.06	+0.72	—1.6	Basal, Asleep
		34.55	20.32	32.04	18.85	110.52	65.0	+1.56	+1.46	+8.3	Basal, Awake
Max W.	1.68	28.32	17.16	27.13	16.14	92.25	54.9	—1.60	—1.25	—1.8	Basal, Asleep 45 minutes
		29.31	17.44	27.63	16.44	95.27	56.7	—1.32	—0.95	+0.0	Basal, Asleep 24 minutes
		31.76	18.90	29.39	17.49	101.70	60.5	+0.14	+0.10	+3.8	Fairly quiet
Max W.	1.71	30.11	17.61	27.75	16.22	86.09	50.3	—1.15	—1.17	—6.4	Basal, Asleep 50 minutes
		32.13	18.79	29.98	17.53	103.27	60.4	+0.03	+0.14	+3.7	Very restless
		33.39	19.52	30.43	17.79	108.13	63.2	+0.76	+0.40	+6.5	Very restless
Max W.	1.75	33.83	19.33	31.77	18.15	100.23	57.2	+0.57	+0.76	+0.5	Basal
		35.04	20.02	32.60	18.62	114.21	65.2	+1.26	+1.23	+8.5	Basal
Max W.	1.85	32.82	17.74	31.30	16.92	93.62	50.6	—1.02	—0.47	—6.1	Basal, Asleep 30 min. (quiet)
		35.71	19.30	33.68	18.21	111.22	60.1	+0.54	+0.82	+3.4	Basal, Asleep 5 min. (quiet)
Edw. T.	1.47										
Edw. T.		30.48	20.74	28.26	19.22	88.14	59.9	+1.98	+1.83	+3.2	Basal, Reading 45 minutes
Edw. T.		42.85	29.15	40.61	27.62	131.13	89.2	+10.39	+10.23	+32.5	Asleep 50 minutes
Edw. T.	1.48	27.56	18.62	23.99	16.21	81.35	54.9	—0.14	—1.18	+1.8	Basal, Reading, quiet
(59 min.)		25.72	17.38	23.88	16.13	84.89	57.4	—1.38	—1.26	+0.7	Basal, Sleeping 55 minutes
Edw. T.	1.47	24.59	16.72	22.82	15.52	69.79	47.5	—2.04	—1.87	—9.2	Basal, Asleep, sleep restless
		29.05	19.76	26.22	17.84	73.36	49.9	+1.00	+.045	—6.8	Reading 55 minutes
Edw. T.	1.47	25.80	17.55	23.71	16.13	76.63	52.1	—1.21	—1.26	—4.6	Basal, Asleep, quiet
		29.12	19.81	26.38	17.95	80.53	54.8	+1.05	+0.56	—1.9	Reading, quiet
		30.48	20.59	28.59	19.45	97.31	66.2	+1.83	+2.06	+9.5	Sleeping
Edw. T.	1.48	31.02	20.96	28.27	19.10	95.83	64.7	+2.20	+1.71	+8.0	Basal, Restless
		33.75	22.80	34.47	23.29	106.76	72.1	+4.04	+5.90	+15.4	Restless
Edw. T.	1.51	31.77	21.04	29.39	19.46	90.04	59.6	+2.28	+2.07	+2.9	Basal, Sleeping, quiet
		33.17	21.97	30.60	20.26	100.06	66.2	+3.21	+2.87	+9.5	Basal, Awake, quiet
Jas. Mgr.	1.32	31.34	23.75	28.78	21.80	87.48	66.3	+4.99	+4.41	+9.6	Basal, Quiet, reading
		30.92	23.42	29.96	22.69	92.07	69.7	+4.66	+5.30	+13.0	Basal, Quiet, reading
Rate of 1 hr.		33.34	25.26	30.54	23.14	103.32	78.3	+6.50	+5.25	+21.6	Fairly quiet
Jas. Mgr.	1.37	32.14	23.46	31.91	23.29	89.64	65.4	+4.70	+5.90	+8.7	Basal, Fairly quiet
		33.34	24.33	31.25	22.81	101.86	74.3	+5.57	+5.42	+17.6	Basal, Fairly quiet
Dr. G. S. L.	1.89	33.19	17.56	31.92	16.89	104.54	55.3	—1.20	—0.50	—1.4	Basal, Quiet
		33.30	17.62	30.88	16.34	102.91	54.4	—1.14	—1.05	—2.3	Basal, Quiet
Peter N.	1.79	22.85	12.76	20.32	11.35	73.79	41.2	—6.00	—6.04	—15.5	Basal, Quiet
		24.71	13.80	21.14	11.81	75.80	42.3	—4.96	—5.58	—14.4	Basal, Quiet
Bessie H.	1.42	19.06	13.42	17.02	11.99	55.89	39.4	—5.34	—5.40	—17.3	Basal, Quiet
		19.41	13.67	16.68	11.74	62.93	44.3	—5.09	—5.65	—12.4	Basal, Quiet
Bessie H.	1.43	18.42	12.88	16.22	11.34	51.06	35.7	—5.88	—6.05	—21.0	Basal, Quiet
		19.49	13.63	17.83	12.47	62.55	43.7	—5.13	—4.92	—13.0	Basal, Quiet
Benny L.	0.83	10.28	12.38	7.67	9.24	28.47	34.3	—6.38	—8.15	—22.4	Basal, Quiet
		10.23	12.33	8.58	10.34	30.74	37.0	—6.43	—7.05	—19.7	Basal, Quiet
Benny L.	0.85	9.44	11.11	7.76	9.13	25.44	29.9	—7.65	—8.26	—26.8	Basal, Quiet
		9.20	10.83	7.83	9.21	26.93	31.7	—7.93	—8.18	—25.0	Basal, Quiet
TOTAL—48			900.51		834.26		2720.4				
Average			18.76		17.39		56.7				

applied to the study of pathological conditions on a large scale without sufficient investigation, it seems to us, into the applicability of methods that were found satisfactory in dealing with healthy subjects to the study of ill patients. We lack precise information upon the relation of oxygen intake and of carbon-dioxide output to the heat production in thyroid diseases, for example, though this is the condition in which accurate gas exchange work is much to be desired. It is possible to determine the accuracy of O_2 and of CO_2 individually as indices to the heat production in hyperthyroidism by an analysis of the standard work of DuBois,[7] who studied a large number of such cases in the chamber calorimeter. It is to be remembered that a study of observations made in the chamber calorimeter will give us results that were obtained under ideal conditions, and when we apply the lessons derived from such work to indirect calorimetry, we must investigate carefully any additional influence that may be present in the particular method under consideration. It seems fair to assume also that any definite finding obtained under the ideal conditions of the chamber calorimeter upon a considerable number of patients will have its bearing under the less favorable conditions of indirect calorimetry, and that we may not expect to obtain substantially better results by indirect than by direct calorimetry.

For the purpose of establishing the caloric equivalents of oxygen and of carbon-dioxide, respectively, in thyroid diseases, I have analysed the DuBois series of cases mentioned above; in this way it was possible to make comparisons between heat production as measured directly, on the one hand, and oxygen, carbon-dioxide, and total indirect calorimetry, respectively, on the other hand.

The series includes observations upon one cretin; otherwise the cases are those of other forms of thyroid disease. The cretin yields figures that are about 20% below those of a normal adult. The essential deviation of the series as a whole is from normal to a hyperthyroid status, and may be used, from the metabolic standpoint at least, as it was used by DuBois—as a study of "Exophthalmic Goiter." The figures are taken from periods of one hour's duration except in two instances, which are noted; these are periods of less than one hour. A total of 48 one-hour periods is obtainable for analysis, being observations upon patients in the "basal" condition, in which the body surface measurements are noted.

Discussion of Figures

In this first table absolute values are not taken into consideration, but the variation of the oxygen from its own average it calculated, and the same is done with respect to the carbon-dioxide and calorie figures. By comparing O_2 variation with calorie variations and CO_2 variations with calorie variations from hour to hour we are able to calculate the total and the average error in

Deviation of CO_2 from Avg. %	Deviation of O_2 from Avg. %	Deviation of Cals. from Avg. %
+12.1	+16.2	+ 6.1
+21.8	+25.7	+28.4
+ 0.9	+ 6.0	—10.9
+14.6	+14.3	+25.9
+11.6	+12.1	+ 9.5
+12.7	+15.9	+17.3
— 0.3	+ 4.1	— 2.8
+ 8.3	+ 8.4	+14.6
— 8.5	— 7.2	— 3.2
— 7.0	— 5.5	0.0
+ 0.8	+ 0.6	+ 6.7
— 6.1	— 6.7	—11.3
+ 0.2	+ 0.9	+ 6.5
+ 4.0	+ 2.3	+11.5
+ 3.0	+ 4.4	+ 0.9
+ 6.7	+ 7.0	+14.9
— 5.4	— 2.7	—10.8
+ 2.9	+ 4.7	+ 5.9
+10.6	+10.5	+ 5.6
+55.4	+58.8	+57.3
— 0.8	— 6.8	+ 3.2
— 7.3	— 7.2	+ 1.2
—10.9	—10.8	—16.2
+ 5.3	+ 2.6	—11.9
— 6.4	— 7.2	— 8.1
+ 5.6	+ 3.2	— 3.4
+ 9.8	+11.8	+16.8
+11.7	+ 9.8	+14.1
+21.6	+33.9	+27.1
+12.8	+11.9	+ 5.1
+17.1	+16.5	+16.8
+26.6	+25.4	+16.9
+24.8	+30.5	+22.9
+34.7	+33.6	+38.1
+25.1	+33.9	+15.4
+29.7	+31.2	+31.0
— 6.4	— 2.9	— 2.5
— 6.1	— 6.0	— 4.1
—31.9	—34.7	—27.4
—26.5	—32.1	—25.4
—28.5	—31.6	—30.5
—27.1	—32.5	—21.9
—31.4	—34.8	—37.0
—27.4	—28.3	—22.9
—34.0	—46.9	—39.5
—34.3	—40.5	—34.8
—40.8	—47.5	—47.3
—42.3	—46.5	—44.1

[7] DuBois, E. F.: The Basal Metabolism in Exophthalmic Goiter. Arch. Int. Med. 1916, XVII, 915.

Deviation of CO_2 from Cals. in Per cent

+ 6.0	+ 5.4	+ 1.9
— 6.6	— 3.0	— 3.4
+11.8	+ 5.0	+ 9.7
—11.3	— 1.9	— 1.3
+ 2.1	— 4.0	— 3.9
— 4.6	— 8.5	— 2.0
+ 2.5	+ 5.3	— 4.5
— 6.3	+17.2	— 1.1
— 5.3	+ 1.7	+ 2.0
— 7.0	+ 9.0	— 5.2
— 5.9	— 7.0	+ 5.6
+ 5.2	— 2.4	— 4.5
— 6.3	— 5.5	+ 5.5
— 7.5	+ 7.7	+ 0.5
+ 2.1	+ 0.3	+ 6.5
— 8.2	+ 9.7	+ 1.8

Total—251.7
Average—±5.2%

Deviation of O_2 from Cals. in Per cent

+10.1	+ 8.1	+ 7.6
— 2.7	— 1.2	— 4.5
+16.9	+ 4.9	+18.5
—11.6	+ 1.5	+ 0.2
+ 2.6	—10.0	— 0.4
— 1.4	— 8.4	— 1.9
+ 6.9	+ 5.4	— 7.3
— 6.2	+14.5	— 6.7
— 4.0	+ 0.9	— 1.1
— 5.5	+ 6.6	—10.6
— 6.1	— 5.0	+ 2.2
+ 4.6	— 4.3	— 5.6
— 5.6	+ 6.8	— 7.4
— 9.2	+ 6.8	— 5.7
+ 3.5	— 0.3	— 0.2
— 7.9	+ 8.5	— 2.4

Total—280.3
Average—±5.8%

the correlation of the curves of O_2 and CO_2 away from the curve of the heat production measured directly in the chamber. Direct and indirect calorimetry figures were treated in the same way. The results are given below.

Average error from direct calorimetry in the curves of O_2, CO_2 and total indirect calorimetry (48 periods).

Average difference between direct calories and CO_2.....	±5.2%
Average difference between direct calories and O_2.....	±5.8%
Average difference between direct and indirect calories.....	±5.8%

In addition to the figures given above, which are purely relative and in which only the correlation of the fluctuations of the various factors from hour to hour was considered, it seemed advisable to compare the figures of DuBois's experiments with some established normal level, in order to calculate the absolute elevation of each of the factors from some base line. It is not essential to this calculation to select the metabolic level of any particular age or sex, but we shall arbitrarily choose for purposes of comparison some well established level of normal at which we may feel safe to conclude that the oxygen, carbon-dioxide and calorie figures are *equivalent* to one another. For this base line we shall take that of normal young men, since there are enough figures in the literature upon such subjects to give reliable average figures.

It is now customarily accepted that 39.5 calories per square meter per hour is the average heat production of such young men. In a previous report I [8] calculated the average carbon-dioxide elimination of healthy young men between the ages of 20 and 30 to be 12.98 gms. per sq. meter per hour. The average oxygen consumption of 62 healthy young men in the same decade, reported by Benedict, Emmes, Roth and Smith,[9] was calculated by me to be 8,076 liters, or 11.54 gms., per sq. meter per hour. On this basis the comparisons are as follows:

	CO_2 per sq. m. per hr. (gms.)	Direct cals. per sq. m. per hour	O_2 per sq. m. per hr. (gms.)
Thyroid cases	18.76	56.7	17.39
Normals	12.98	39.5	11.54
Elevation of thyroid cases above normal.....	5.78	17.2	5.85
Elevation of thyroid cases above normal in %	+44.5	+43.6	+50.7

In other words the average elevation of the CO_2 elimination in the 48 case-hours cited was 44.5%, or 0.9% higher than the average elevation of calories by direct measurement. The average elevation of oxygen was 7.1% above that of the direct calories.

A study of the work of Benedict, Emmes, Roth and Smith, noted above, shows the average CO_2 elimination of 62 healthy men in the third decade to be 13.13 gms. per sq. meter per hour. If we use this figure our comparisons become:

	CO_2 per sq. m. per hr. (gms.)	Direct cals. per sq. m. per hour	O_2 per sq. m. per hr. (gms.)
Thyroid cases	18.76	56.7	17.39
Normals	13.13	39.5	11.54
Elevation of thyroid cases above normal.....	5.63	17.2	5.85
Elevation of thyroid cases above normal in %	+42.9	+43.6	+50.7

[8] *loc. cit.*

[9] Jour. Biol. Chem., 1914, XXII, No. 2.

Another standard figure for heat production of young men may be obtained by computing the caloric equivalent of the oxygen consumed per sq. meter per hour, as obtained by Benedict and his associates. This figure, 8,076 c.c., is used above to represent the average oxygen consumption for this decade; from the Lusk [10] modification of the Zuntz and Schumburg table of caloric equivalents, and accepting 0.82 as the average normal respiratory quotient, we find the corresponding heat production to be 39.0 calories per sq. m. per hour. We can now establish oxygen, carbon-dioxide and calorie figures for the identical group of subjects. Compared with these standards the figures for the thyroid cases are seen to be as follows:

	CO_2 per sq. m. per hr. (gms.)	Direct cals. per sq. m. per hour	O_2 per sq. m. per hr. (gms.)
Thyroid cases	18.76	56.7	17.39
Normals	13.13	39.0	11.54
Elevation of thyroid cases above normal.....	5.63	17.7	5.85
Elevation of thyroid cases above normal in %	42.9	45.4	50.7

From this it is seen that the average elevation of the oxygen figure is 5.3% above that of direct calories, while the elevation of CO_2 is 2.5% below that of the direct calories.

By using several different standards, therefore, we arrive at the same result, namely, that the oxygen consumption in thyroid disease is relatively higher than heat production and carbon-dioxide elimination. The absolute carbon-dioxide figure and the absolute calorie figure correspond so closely, and the oxygen intake is so definitely elevated in proportion to these two, that there is distinct need of proof that oxygen consumption (or rather *absorption*) is a reliable index to the heat production and total metabolism in hyperthyroidism, unless the discrepancy depends upon technical error in chamber calorimetry.

DuBois found that the total calorimetry of this series by the indirect method was 2.9% higher than that by the direct method. This difference is due to the fact that the indirect calorimetry is based primarily upon the oxygen intake, which is higher in this series. We shall return to this subject in the discussion at the end of the paper.

Various considerations might lead us to prefer one or another of the three groups of standard figures used for the above comparisons. They are all based upon a considerable number of observations by both direct and gas exchange methods, and we should probably arrive at the approximate truth by taking the average of the three comparisons. At the same time the average figure for indirect calorimetry, which I have calculated for the 48 hour experiments under consideration, will be compared with that of direct calorimetry.

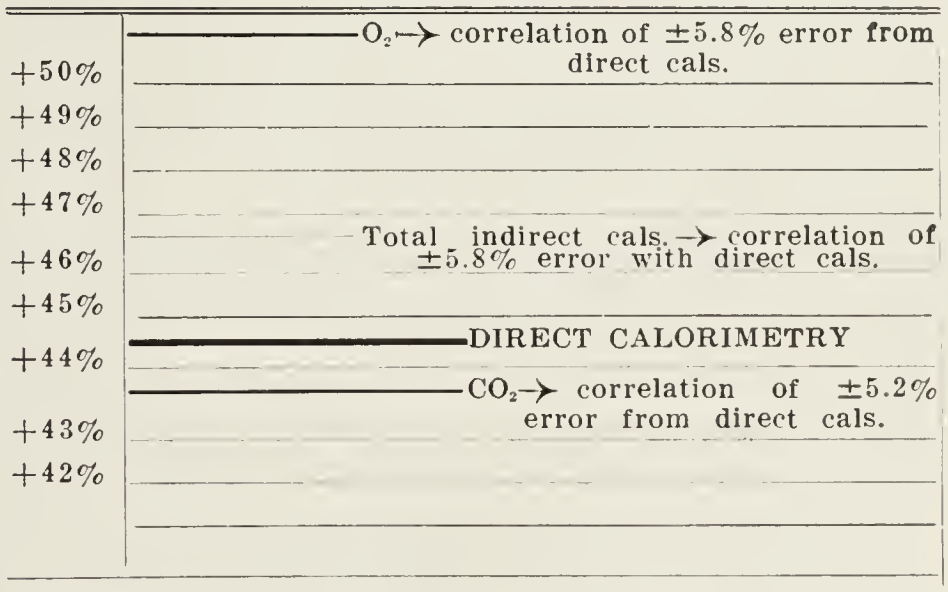
[10] *loc. cit.*

	Variation of O_2 from direct calories	Variation of indirect from direct cals.	Variation of CO_2 from direct cals.
1st Comparison	+7.1%		+0.9%
2nd Comparison	+7.1%		—0.7%
3rd Comparison	+5.3%		—2.5%
Average	+6.5%	+2.1%	—0.8%

Table showing the average variation in % from direct calorimetry of O_2, indirect calorimetry and CO_2, respectively. The average elevation of the various factors was computed from 48 one-hour observations upon cases of thyroid disease (DuBois) and compared with 3 sets of standard figures.

We have now considered separately the two comparisons that are of interest in investigating the caloric equivalents of oxygen, carbon-dioxide and indirect calorimetry (which is analogous to the "Tissot" method) in hyperthyroidism. These two comparisons are: (1) comparison of these various factors with direct calorimetry without consideration of the relation of any of them to normal figures; and (2) comparison of the average elevation in thyroid disease of each of these factors, and of the direct calories as well, away from a normal level. In the first comparison CO_2 varies from direct calorimetry less (±5.2%) than is the case with either O_2 or total indirect calorimetry (±5.8%, respectively). In the second comparisen CO_2 varies from direct calories only—0.8% as compared with +6.5% for O_2 and +2.1% for the total indirect calorimetry. The two comparisons may be combined and considered schematically as follows:

CHART I.



Schema showing the elevation of the various factors above a normal base line and the correlation error that is to be expected for each factor with direct calorimetry in addition to the absolute elevation of each. Based on the observations of DuBois noted above and compared with 3 sets of normal figures.

Discussion

One hesitates to draw too definite conclusions from work with the technique of which he has not had personal experience. Direct calorimetry is a technical luxury that

few of us have enjoyed. Yet the publications of the Russell Sage Institute are of such importance that they deserve the closest study by clinicians. Moreover, it is naturally impossible for DuBois and his associates to investigate for us all the matters with which we, as physicians, may have concern.

It seems to us, however, that the results of the analysis are clear enough to warrant certain deductions. Carbon-dioxide elimination is more closely associated with heat production, as measured directly by DuBois, than is O_2 absorption. This at once leads to the conclusion that the theory of caloric equivalents as expressed by the table of Zuntz and Schumburg cannot be applied literally to the gas figures of chamber calorimetry. It is equally certain that the caloric equivalent of CO_2 would not be so constant unless the actual respiratory quotient were constant or very nearly constant.

In this connection we may quote Benedict and Tompkins:[11] "To be capable of a significant interpretation, the respiratory quotient should deal not with the carbon-dioxide *excretion* and oxygen *absorption* during a given time, but with the carbon-dioxide *production* and oxygen *consumption*." It seems obvious that the somewhat low "respiratory quotient," (the average is 0.792), that was found in this series of thyroid cases of DuBois and that is so often met with clinically cannot be due to a lowered CO_2 production, for the CO_2 figure is strikingly constant at the level of actual heat production. It is apparently caused by an elevation of the O_2 figures to a level relatively higher than that of the heat production.

In other words, there exists apparently a discrepancy between the measured gas exchange at the mouth, called by Benedict and his school the "gaseous ratio," and the actual respiratory quotient $\left(\frac{\text{Vol. } CO_2}{\text{Vol. } O_2}\right)$ actually concerned with metabolic processes during the periods in question.

We are dealing in thyroid diseases, then, with an altered "gaseous ratio," calculated by DuBois for all his cases to be about 0.77. As stated above, it seems to us unlikely that this is to be explained by an absolute reduction in CO_2 since the CO_2 level was only 0.8% below that of measured calories. Moreover, the correlation of CO_2 production with heat production is more nearly parallel than is that of O_2 with heat production. The outstanding feature of the analysis is the elevation of O_2 above all other factors under consideration, and it seems reasonable to explain the low gaseous ratio on the basis of the high oxygen measurement. Moreover, if the amount of O_2 *metabolized* during these tests were the same as the O_2 *absorbed,* we know by Zuntz and Schumburg's table that the heat production would be higher than that which was measured in this series of cases. DuBois noted the slight difference between direct calorimetry and indirect calorimetry in these experiments and considered that it might be due to an error in direct calorimetry—to a loss of heat. He noted the same phenomenon in observations upon typhoid fever. If we glance at Chart I we see that CO_2 is still a better index to heat production than is O_2, even though we correct the direct calorimetry by assuming it to be incorrect and raising it to the level of indirect calorimetry.

There is abundant literature bearing upon O_2 absorption and CO_2 elimination, much of which came out of observations upon aviators in the World War. As a result of extensive experimental observations upon a scale never approached before it was concluded that "CO_2 in the blood is the stimulant which excites the nervous center (respiratory center) of our respiratory mechanism and maintains the regular action."[12]

Such considerations lead us to the conclusion that CO_2 is eliminated promptly as a result of body metabolism and that it acts directly upon the respiratory center.

Finally, in connection with the use of O_2 as an index to heat production in hyperthyroidism, it is well to remember that Benedict, speaking of the "portable apparatus" that bears his name, names this conservative statement:[13] "This laboratory has at no place or time recommended the portable apparatus for the diagnosis of or as an index of the treatment for any specific disease."

An analysis of the O_2, CO_2 direct and indirect calorimetric figures for the successive hours of the experiments may be made. These figures were computed and found to be as follows:

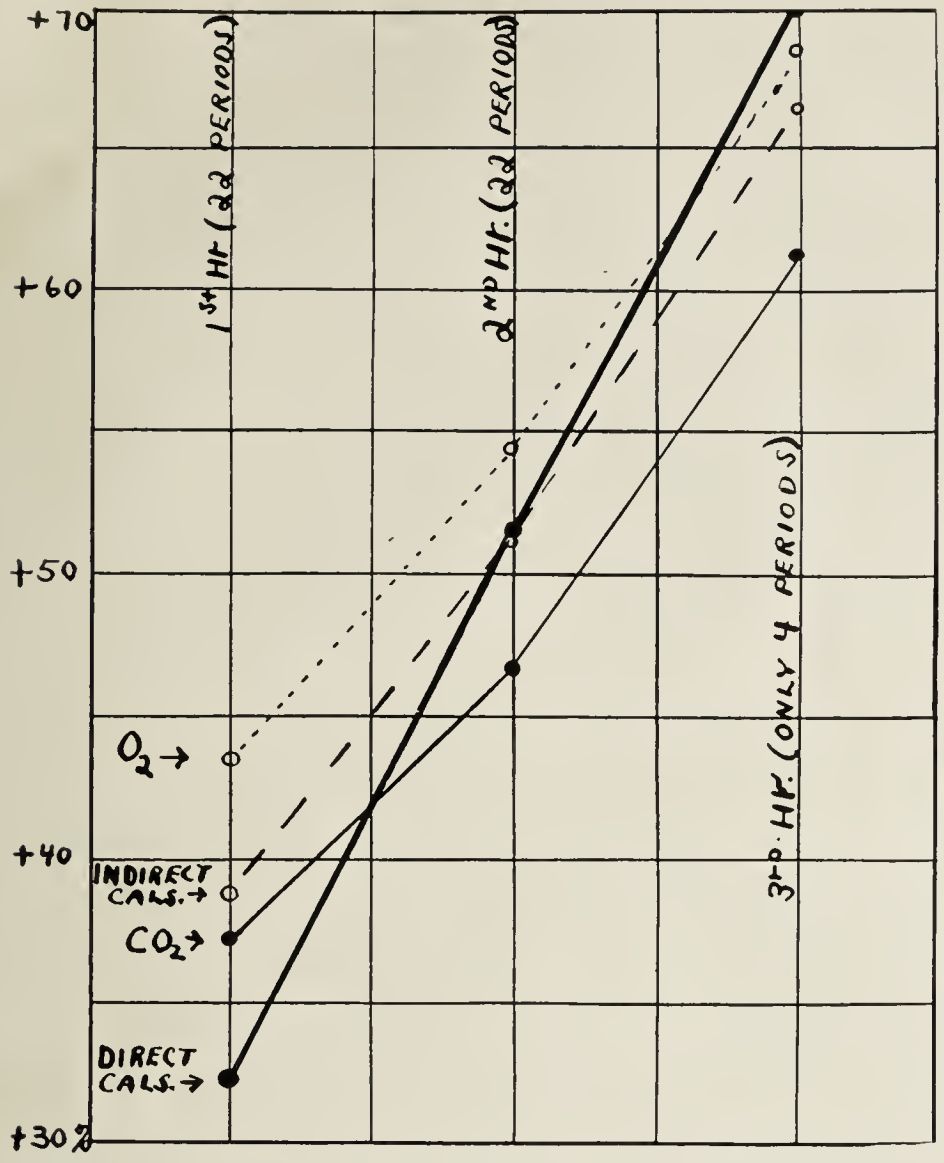
	Per sq. m. per hr. O_2	Per sq. m. per hr. CO_2	Per sq. m. per hr. direct calories	Per sq. m. per hr. indirect calories
1st hour—(22 cases)—				
Thyroid cases	16.56	17.93	52.1	55.3
Normal standards	11.54	13.08	39.3	39.3
Deviation from normal in %	+43.5	+37.1	+32.8	+38.9
2nd hour—(22 cases)—				
Thyroid cases	17.82	19.17	59.3	59.0
Normal standards	11.54	13.08	39.3	39.3
Deviation from normal in %	+54.4	+46.6	+50.9	+50.5
3rd hour—(4 cases)—				
Thyroid cases	19.47	21.07	67.0	64.4
Normal standards	11.54	13.08	39.3	39.3
Deviation from normal in %	+68.7	+61.1	+70.3	+66.4

This may be plotted in a curve as follows:

[11] Benedict and Tompkins: Boston. Med. and Surg. Journal, 1916, CLXXIV, 863.

[12] Air Service, Medical, War Department, Washington, 1919.

[13] Benedict, F. G.: Notes on the use of the portable respiration apparatus. Boston, Med. and Surg. Jour., 1920, CLXXXII, 253.



Analysis of 48 one-hour observations of thyroid cases. (DuBois, Chamber Method.)

SUMMARY

1. The average results of 48 one-hour observations upon patients with thyroid disease in the "basal" state showed:

   a. The average CO_2 elevation is 0.8% less than the elevation of direct calories, and there is an average error in correlation with direct calories from hour to hour of ±5.2%.

   b. The average elevation of indirect calories is 2.1% above direct calories for those experiments. The error in the correlation of direct and indirect calorimetry from hour to hour is ±5.8%.

   c. The average elevation of O_2 is 6.5% above the direct calories, and the average error in correlation from hour to hour is ±5.8%.

2. The average gaseous ratio is 0.791 in the first hour and 0.796 in the second hour. In the third hour (4 observations) it is .0767.

3. In observations upon quiet patients the gaseous ratio falls in the second hour in 11 cases and rises in 16 cases. In restless patients it falls in 5 cases and rises in 6 cases.

CONCLUSIONS

1. CO_2 elimination affords the best index to the heat production in thyroid disease, as judged by direct calorimeter experiments.

2. Indirect calorimetry by the chamber method is a trifle less accurate than CO_2 as an index to direct calorimetry. This is doubtless the effect of the O_2 figures in the calculation of calories, or due to inaccuracies in the direct heat measurement.

3. While basal metabolism estimations by gas exchange methods are usually done for shorter periods than one hour we have no shorter chamber calorimetric experiments to guide us. It is felt that the hour observations will give us a guide to what we may expect to find in shorter periods, provided no other factor supervenes.

4. Bearing in mind the technical errors inherent in metabolism experiments by the direct method, the good correlation of CO_2 elimination with direct calorimetry shows that CO_2 is a fair index to heat production in hyperthyroidism and that the value of CO_2 as an index to heat production is not significantly altered by the slight reduction of the respiratory quotient in hyperthyroidism.

THE DETERMINATION OF CORPUSCULAR VOLUME FROM THE RELATIVE VISCOSITIES AND CONDUCTIVITIES OF BLOOD AND PLASMA OR SERUM

By B. S. NEUHAUSEN and Z. T. WANG

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A number of investigations have been made to find a relation between the corpuscular volume of a blood sample and some physical properties of the whole blood and the plasma or serum. Since the red blood corpuscles are very bad conductors, the idea suggested itself to a number of investigators that there must be a relation between the conductivity of the blood and serum and the relative volume of corpuscles and serum. Thus Bugarsky and Tangl [1] on the basis of a small number of experiments proposed the formula

$$p = 92 \frac{\lambda(b)}{\lambda(s)} + 13$$

where p is the percentage volume of serum, $\lambda(b)$ the conductivity of the blood and $\lambda(s)$ the conductivity of the serum. They did state, however, that had they made a larger number of observations, they very probably would have had to modify their formula.

Stewart [2] obtained the following empirical formulae which gave values for the percentage serum that agreed rather satisfactorily with Hoppe-Seyler's method and his own colorimetric method:

$$p = \frac{\lambda(b)}{\lambda(s)} (180 - \lambda(b) - \sqrt{\lambda}) \qquad (2)$$

or

$$p = \frac{174.5\ \lambda(b) - \lambda^2(b)}{\lambda(s)} \qquad (3)$$

The reason for the rather complicated form of Stewart's serviceable formulae becomes apparent when we consider that the corpuscles do not decrease the conductivity of the blood merely by occupying a portion of the volume with a bad conductor but they also increase enormously its viscosity. Since the conductivity of a solution, other things being equal, varies usually as some fractional power of the viscosity of the medium, it may be seen that if we express everything in terms of percentage serum or percentage corpuscles the conductivities of the blood must be given in several powers. It was believed by the writers that a formula less empirical in nature could be obtained if the viscosities of the blood and plasma were taken into account.

The following considerations were employed in deriving the formula presented below. We assumed that the electrolytes of the plasma of whole blood were in the same condition as in the separated serum, as was found in the case of Na and Cl by Neuhausen and Marshall.[3] Since the corpuscles are poor conductors, only a portion of the volume is actually conducting and therefore to obtain what would be the conductivity of the blood, if it were to have the same conducting volume as an equal volume of plasma, it should be divided by 1—percentage volume of corpuscles. The non-conducting volume having been corrected for, it was believed that the product of the corrected specific conductivity of the blood times some fractional power of the viscosity would equal that of the specific conductivity of the serum times its viscosity raised to the same fractional power. By trial the one-third power was found to give most satisfactory results. The formula we propose is

$$\frac{\lambda(b)\ (n_b)^{1/3}}{1 - c.v.} = \lambda(s)\ (n_s)^{1/3} \qquad (4)$$

or

$$c.v. = 1 - \frac{\lambda(b)\ (n_b)^{1/3}}{\lambda(s)\ (n_s)^{1/3}} \qquad (5)$$

in which $\lambda(b)$ and $\lambda(s)$ are the specific conductivities of the blood and serum, respectively; n_b and n_s—the relative viscosities of the blood and serum, water being taken as unity, and c.v. is the percentage corpuscular volume.

In Table I are presented some of the conductivity results obtained on pig's and dog's blood and serum samples, the first seven samples being taken from normal dogs, the next twelve from pigs and the last seven * from partially nephrectomized hypertensive dogs. The viscosities were determined by means of an Ostwald viscosimeter. The corpuscular volume calculated according to the formula presented above (equation 5) is compared with that calculated by Stewart's first formula (equation 2), the conductivities having been reduced to the value at 5°C., as required by that formula, and also with the volume found by a haematocrite which was centrifugalized at an R.P.M. of 12000, and which after centrifugalization for five minutes showed no further change in volume.

As has been pointed out by Stewart,[4] the corpuscular volume obtained by means of the haematocrite varies with the length of centrifugalizing time and the value calculated from the electrical method constitutes a limit toward which the haematocrite readings are approaching. It will be noticed that with the high speed centrifuge there are often obtained smaller values than those calculated on the basis of Stewart's conductivity formula.

TABLE I.

Temperature of measurement	Specific cond. of blood	Viscosity of blood	Specific cond. of serum	Viscosity of serum	Corpuscular volume calcuated by (5)	Calc. according to Stewart's formula (2)	Haematocrite reading
21	.00420	4.55	.01160	1.63	50	47.3	43.5
21	.00444	4.61	.01180	1.57	46.1	45.7	42.0
21	.00516	4.45	.01280	1.36	40.1	40.3	38.0
18	.00511	4.51	.01155	1.50	36.2	39.9	38.0
20.5	.00518	4.68	.01226	1.53	38.7	42.0	42.5
22.5	.00591	3.97	.01355	1.53	40.1	41.0	38.5
21	.00549	4.18	.01241	1.61	39.0	39.7	39.0
21	.00508	4.24	.01240	1.64	43.8	43	41.5
20	.00467	4.51	.01260	1.61	47.8	47.6	41.0
22	.00412	4.63	.01198	1.63	51.4	49.0	39.0
22.5	.00443	4.55	.01172	1.56	45.9	44.8	42.2
22.5	.00445	4.50	.01116	1.52	42.7	42.0	42.0
21	.00520	3.71	.01228	1.58	43.8	41.2	30.0
20	.00494	4.52	.01305	1.72	47.8	47.1	41.0
22	.00477	4.45	.01197	1.63	44.3	44.4	40.0
15.5	.00487	5.04	.01087	1.74	36.3	41.8	37.0
18	.00439	5.00	.01272	1.63	49.9	51.4	42.5
23.5	.00504	4.55	.01255	1.73	44.5	42.8	41.5
22	.00503	4.47	.01199	1.65	41.5	37.7	40.5
22	.00603	4.54	.01348	1.43	34.2	39.9	34
22	.00644	4.29	.01335	1.63	33.4	36.8	35
23.5	.00546	4.70	.01285	1.35	35.6	40.6	36
20	.00608	4.32	.01195	1.64	29.8	33.4	34
22	.00713	3.64	.01441	1.46	32.9	37.7	35
22	.00480	4.77	.01419	1.51	50.4	39.1	43.5
22.5	.00691	4.41	.01324	1.55	26	33.2	34.0
17	.00596	4.24	.01141	1.64	28.3	33.7	30.0
17	.00539	4.42	.01182	1.51	34.5	38.2	32.0

This fact may be due to a compression of the biconcave elliptical cells into more closely packed spheres with a consequent diminution in volume. Whether the compression be the true explanation or not, it seems justifiable to consider the haematocrite reading obtained by us as some function of the true corpuscular volume.

* These seven blood samples were furnished by Dr. A. C. Kolls.

From a comparison of columns 6, 7 and 8, it will appear that on the whole the formula presented above is as satisfactory as that of Stewart. It has the advantage of containing only terms with a physical significance and to that extent is free of empiricism.

Attention should also be called to the fact that the formula applies equally well to the bloods and sera of the hypertensive dogs.

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A STUDY OF THE GROWTH OF CERTAIN MICROORGANISMS ON REINOCULATED MEDIA

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The growth curve of many bacteria has been carefully studied by various investigators and found to follow a general law; namely, initial lag, logarithmic increase, stationary and decrease periods. The reason for this cyclic variation in the rate of growth has been largely speculative. However, Barber[1] and McKendrick and Pai[2] have shown that the initial lag period may be obliterated if young organisms are used for the inoculum. The logarithmic increase rate is perhaps a natural seqnence of unrestricted cell division; but the reason for the stationary growth period and also the final decline is problematical. Many ideas have been developed to explain this stationary period of the growth curve as well as the decrease phase, such as acid production, destruction by metabolic products, exhaustion of available food supply, etc. Before beginning an investigation in the hope of throwing some light on the problem of the growth curve, we shall make a preliminary report on the study of the last mentioned assertion, namely, the exhaustion of the food supply; especially with reference to nitrogen and carbohydrate metabolism of bacteria. It is the purpose of this study to ascertain whether or not the available food supply is depleted by growing microorganisms from a standard meat extract broth.

That the food requirements for the vegetation of bacteria are not completely consumed by a single growth of an organism has been shown by several workers. Marmorek,[3] studying streptococci, claimed that medium upon which this organism had been grown would not support a second growth of the same organism; but pneumococci grew well in this used medium. However, Brown,[4] using perhaps better technique, could not repeat this specific phenomenon and found that certain streptococci grew on the reinoculated plate, or to use his terminology, on "metabolized media." More recently, Graham-Smith[5] has clearly proven that media on which an organism had grown once would support a second growth. Juth,[6] making use of the fact that organisms will grow on resterilized, rejuvenated medium, describes a method for the repeated use of Endo's and Drigalski's agar.

EXPERIMENT I

Sterilization by Autoclave

The first experiment was carried out in the following manner: ordinary meat extract broth was prepared in two-liter lots,—1 per cent. peptone (Difco), 0.5 per cent. sodium chloride and 0.2 per cent. meat extract, titrated to pH 7.8. The flasks of medium were inoculated with the number of organisms on an 18-hour agar slant and allowed to incubate for four days, at the end of which time the greater number of the organisms were thrown down in a Sharples centrifuge and the fluid run through a Mandel filter. The medium was re-titrated and re-autoclaved for a half-hour at 17 pounds pressure and then re-inoculated and incubated for another four days. This procedure was repeated until no growth occurred during the four-day period of incubation.

Four different organisms were studied: B. lactis aerogenes, B. coli communis, B. pyocyaneus and B. alkaligenes. The B. lactis aerogenes was a strain of organism isolated from the blood stream of a patient dying from broncho-pneumonia, and at the time of the experiment was of such virulence that as few as 20 organisms caused the death of a cat in 24 hours, when injected into the subarachnoid space. The other organisms used had been recently isolated from patients coming to autopsy. They were typical organisms by the ordinary tests. B. pyocyaneus fermented dextrose and glycerine slightly. Stains were made from the growth of each before inoculation and also appropriate sugars were inoculated as a means of insuring the use of pure cultures.

TABLE I.

Addition of the Different Constituents of Media Sterilization by Autoclave.

Microorganism	No. of Growths	Peptone			Meat Extract			NaCl 0.3%	Tyrosin
		1%	0.5%	0.2%	0.1%	0.05%	0.01%		
B. lactis aerogenes	7	++++	++++	++	++	+	+	±	+
B. coli communis	6	++++	++++	++	++	+	+	±	+
B. pyocyaneus ...	5	++++	++++	++	++	+	+	±	+
B. alkaligenes ...	5	++++	++++	++	++	+	+	±	+

Table I shows the number of times it was possible to obtain a definite growth with the different microorganisms investigated. For the second and third inoculations there was practically the same number of organisms as in the first growth. Calculations of numbers were based on a standard turbidity: a 24-hour growth of each organism on fresh media taken as a four plus (++++). From the fourth inoculation there was observed a gradually diminished growth until the last, when no increase of turbidity could be detected. Inasmuch as we wished to learn whether or not the food supply was entirely exhausted by growth of these microorganisms, the possibility that some of the food for the subsequent bacterial growth may have been supplied by autolized bacteria, although a complicating factor, does not vitiate our experiments.

Having exhausted the medium so that bacteria did not multiply, we were in a position to test the nature of the substance exhausted whether it be nitrogen or carbohydrate. To different batches of the media, exhausted by the varying microorganisms, were added varying percentages of peptone, meat extract and sodium chloride; the materials used being taken from the same lot from which the original sample had been prepared. The results of the experiment are shown in Table I. As might be expected, the sodium chloride did not restore any of the food necessary for the multiplication of bacteria. However, the meat extract in one-half the concentration used in making the original media did support growth, but only to a slight extent. With peptone the results were quite different, 1 per cent, and 0.5 per cent. seemingly restoring all the food elements necessary for optimal development. It would appear, therefore, that peptone contains the requisite food elements to restore the worn-out medium and that the available nitrogen was the substance which had been exhausted. That this is not necessarily true is shown below.

In the same manner as with peptone and sodium chloride, six of the ordinary carbohydrates—1 per cent. dextrose, maltose, lactose, saccharose and mannite and 5 per cent. glycerine—were added to small portions of the old medium and were inoculated with the respective microorganisms. This experiment is tabulated in Table II. Briefly, with each bacterium, optimal growth occurred

TABLE II.

Addition of Carbohydrates
Sterilization by Autoclave.

Microorganism	Dextrose 1%	Maltose 1%	Saccharose 1%	Mannite 1%	Glycerine 1%
B. lactis aerogenes	++++	++++	++++	++++	++++
B. coli communis .	++++	++++	+	++++	++++
B. pyocyaneus	+++	+	+	+	++
B. alkaligenes	+	+	+	+	+

in the carbohydrates which ordinarily were oxidized by these microorganisms. B. lactis aerogenes grew abundantly with all the carbohydrates, while B. alkaligenes showed only a faint growth. B. coli communis grew well on all the carbohydrates except saccharose. Although B. pyocyaneus was capable of multiplication after the addition of glycerine and dextrose—two carbohydrates which this organism ferments normally—to the exhausted medium, optimal growth did not occur; no doubt owing to its inability to utilize a sufficient amount of this form of carbohydrate.

The fact that either peptone or a carbohydrate which was normally oxidized by a specific microorganism revived the growing condition for these bacteria made impossible a decision as to whether the carbohydrate or the nitrogen had been exhausted. To answer this question, tyrosine (a saturated solution) was added to the different lots of metabolized medium and inoculated as above. Slight growth occurred with the different organisms, no more than a single plus (+): the degree of growth being in no way comparable to that obtained when peptone or carbohydrate was added to the exhausted medium.

Experiment II

Sterilization by Berkefeld

A second experiment was performed in which sterilization between inoculations was done by Berkefelding the medium instead of autoclaving. The procedure consisted in inoculating the several lots of medium with the bacteria as described above, allowing them to grow for four days, and then after centrifugalization and re-titration, passing the supernatant fluid through a Berkefeld candle. The results in the main were like those of the former experiment in which sterilization of the metabolized medium was effected by autoclaving. As can be seen from Table III, however, growth did not occur for so many

TABLE III.

Addition of the Different Constituents of Media
Sterilization by Berkefeld

Microorganism	Number of Growths	Peptone 1%	Meat Extract 0.1%	Tyrosine Saturated
B. lactis aerogenes . .	4	++++	++	+
B. coli communis . . .	3	++++	+	+
B. pyocyaneus	4	++++	+	+
B. alkaligenes	4	++++	+	+

inoculations as in the medium sterilized by autoclave. It is interesting to note that B. coli gave poorer growth on reinoculation than did the other organisms studied. The fact that growth of the four organisms was not supported for so long a time on the metabolized medium sterilized without heat indicates that autoclaving either destroys some inhibiting substance or increases the amount of available food supply. We may assume, however, that

inhibiting sustances do not exist in great concentration; for, as seen from the results reported in Table III, with the addition of peptone or carbohydrate, (Table IV)

TABLE IV.

Addition of Carbohydrates
Sterilization by Berkefeld.

Microorganism	Dextrose 1%	Maltose 1%	Saccharose 1%	Mannite 1%	Glycerine 5%
B. lactis aerogenes	++++	++++	++++	++++	++++
B. coli communis	++++	++++	+	++++	++++
B. pyocyaneus....	+++	+	+	+	++
B. alkaligenes....	+	+	+	+	+

maximal growth is obtained. No doubt, therefore, heating insures a greater supply of food, or food which is more availale for bacterial consumption.

DISCUSSION

We may infer from the results obtained in our experiments that the transition from the logarithmic rate of multiplication of microorganisms to the stationary phase is not caused by the lack of food substances in the medium. We do not conclude at present that the available food at the end of the logarithmic curve is or is not sufficient for continued growth at the same rate. There is a possibility that the manipulation necessary in performing the experiments here reported caused such alterations in the medium that the food elements became more suitable for consumption by bacteria, and thus there may have been a temporary depletion of available food substances at the end of the logarithmic phase. Indeed, according to the work of Graham-Smith,[5] there is exhaustion of food after growth has continued for several days. This author has shown in a very convincing manner that media added to cultures of various organisms during their decrease phase cause either a subsequent rise in numbers of organisms or a retardation in rate of decline, depending upon the amount of medium added. He concludes from his experiments that no metabolic toxic products are developed by young organisms, but that there simply occurs a depletion of food substances. However, the data accumulated by this investigator have thrown little light on the reason for the alteration in the growth curve after it has reached its peak, because the addition of fresh food supply was made during the stationary or decrease phase. Further work must be done to decide the problem in reference to the cause of the decrease rate of multiplication of bacteria grown *in vitro*. It is our impression that the transition from the logarithmic increase rate to the stationary phase is not due to exhaustion of media, but to a saturation of living organisms in the fluid medium. The subsequent decline in the multiplication rate may possibly be caused by a transitory lack of food substances.

From our work, we feel justified in assuming that media exhausted of their food supply by growth of the organism used in this study were depleted of their available carbohydrates, in contradistinction to the nitrogen supply. For, whereas with the addition of carbohydrate to the metabolized media which would not support bacterial growth, optimal increase in numbers occurs, the addition of nitrogen in the form of tyrosine did not supply the missing substance; while tyrosine alone furnishes sufficient nitrogen for the growth of the organisms here studied. The reason for restoration of the food elements necessary for growth by the addition of peptone is not quite clear. The presence of a carbohydrate radical in peptone may possibly have furnished the needed carbohydrate which had been exhausted from the media and be responsible for reviving the used media by this substance. Although our method of study and approach differed from that of Graham-Smith, in the main the results reported above confirm his work. It is noteworthy that Avery and Cullen[7] have shown in their studies on pneumococci that if to media, in which this microorganism had grown, giving a definite pH, they added a little dextrose solution, growth continued and the pH lowered; showing that the nitrogen had not been exhausted, while either available carbon or oxygen, or both, had been.

Summary and Conclusions

I. Ordinary meat extract broth was exhausted of its food supply by repeated inoculation and growth of four different bacterial species, B. coli, B. lactis aerogenes, B. alkaligenes and B. pyocyaneus. Sterilization between inoculations was accomplished either by centrifugalization or autoclaving or by passing through a Berkefeld filter. Before steriliazation, the medium was re-titrated to a pH 7.8.

II. The number of times used media could be utilized for food by the various bacteria differed with the different species and also with the method of sterilization. Sterilization by heat seemed to render the food supply of metabolized media more available for bacterial consumption.

III. It was found, regardless of the method of sterilization, that the exhausted media were revived by the addition of peptone or a carbohydrate which could be oxidized by the specific organism; but that complete restoration did not follow the addition of tyrosine, meat extract or sodium chloride.

IV. Under the conditions of the experiments, we assume that the carbohyrate content of the media employed is the first element used up by growing organisms, and accordingly is the constituent necessary to revive the growth-promoting character of media so exhausted.

V. The carbohydrate required to revive the exhausted media is one which is normally oxidized by the microorganism in question: B. alkaligenes, normally being unable to oxidize any of the five carbohydrates employed, showed no increase in growth on the addition of these sugars to exhausted media.

VI. We assume that the transition in the rate of multiplication in the growth curve from the logarithmic increase phase to the stationary phase is not caused by depletion of food supply.

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THE RÔLE OF THE LYMPHATICS IN THE ABSORPTION OF BILE PIGMENT FROM THE LIVER IN EARLY OBSTRUCTIVE JAUNDICE

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Mechanical occlusion of the common bile-duct, whether produced experimentally in animals or whether arising in persons suffering with calculous, inflammatory, or neoplastic disease of the biliary tract, results in jaundice. The commonly accepted view on the manner of origin of the jaundice is that after occlusion of the common bile-duct the bile continues to be excreted by the liver parenchyma, and since it is unable to follow its normal course to the duodenum it is dammed back into the delicate bile capillaries. The increase in tension in these very fine structures causes them to rupture; the extravasated bile is absorbed either by the blood capillaries or by the lymphatics and is then distributed throughout the body, being in part deposited in the tissues and in part excreted with the urine.

Notwithstanding the widespread, deeply rooted belief that the first step in the production of obstructive jaundice is the rupture of the slender intercellular bile-capillaries, it is surprising to find so few reports on the condition of the canaliculi in early obstructive jaundice in animals or persons. The first work of importance on the condition of the capillaries in obstructive jaundice is that of Eppinger,[1] who reports that, in the livers of patients suffering with long-standing mechanical jaundice, the normally thin canaliculi are greatly distended and tortuous and show evidences of frequent rupture into what he calls the pericapillary lymph spaces. Abramov and Samoilowicz,[2] on a long series of livers from jaundiced patients, confirmed Eppinger's results. Jagič,[3] from a series of experiments in which he ligated the common duct in dogs, reports that up to the fifth or sixth day the bile canaliculi are not ruptured, although the animals exhibited scleral jaundice. Ogata [4] reports that in an extensive series of experiments performed on many animals—including the dog—definite jaundice may be present before any ruptured bile canaliculi are to be found.

The question as to whether the bile pigment which is distributed over the body is removed from the liver by way of the lymphatics or by the blood capillaries is a still unsettled one. Fleischl [5] Kunkle [6] and Kufferath,[7] in their experiments on the mechanism of obstructive jaundice, tied the common duct and then found bilirubin, by the Gmelin test, or bile acids, in the lymph stream before they were found in the urine. Harley [8] claimed that after occlusion of the common bile-duct and simultaneous ligation of the thoracic duct, jaundice did not develop in from five to eighteen days in animals in which there were no accessory lymphatic channels leading to the large veins of the thorax. All of the above authors are in favor of the absorption of the bile from the liver by way of the lymphatics. On the other hand, Wertheimer and Lepage [9] and Mendel and Underhill,[10] from experiments in which they injected various substances into the common duct towards the liver, conclude that absorption from the liver is by way of the blood-stream, since they detected these substances in the urine before they appeared in the lymph of the thoracic duct. As late as 1911, Whipple and King [11] concluded from a series of experiments that "It seems clear that in both acute and chronic obstructive jaundice the lymphatic apparatus takes no essential or active part in the absorption of the bile pigments from the liver. At best the lymphatic system is a secondary factor in the mechanism of jaundice."

The apparently contradictory results of the various workers on this problem can be explained in part by taking into account the different kinds of experiments which they performed. The probable sources of error in the previous experiments were the following:

(1) In none of them was the blood examined for the presence of bilirubin at short intervals after the ligation of the common duct. The urine, however, was examined in all cases. While it is true that the bilirubin threshold of the dog's kidney is very low, this procedure may be a

source of error. This point is well illustrated in the case of patients suffering from certain diseases in which, although there is a definite icterus, the bilirubin content of the serum being well above the human renal threshold of 1-60,000, there is not a bilirubinuria but a urobilinuria.

(2) The tests for bilirubin used by the previous workers were the Gmelin and the Salkowsky. The former is not as delicate as later tests, since it is sensitive only to a dilution of 1-80,000; nor is it readily applicable to solutions containing proteins. The latter test cannot be used successfully for traces of bilirubin unless large quantities of serum are available.

(3) The results of the injection of various dyes and other substances into the ductus choledochus cannot be interpreted as reproducing very closely the process of mechanical jaundice.

In view of the unsettled status of this question, a series of experiments was carried out in which both kidneys were removed from a dog in order to prevent any of the bile pigment from being excreted into the urine. The common bile-duct was then doubly ligated and sectioned. The gall-bladder was prevented from functioning, either by complete extirpation or by ligation of the cystic duct, in order to eliminate the variations in the time of appearance of bile in the blood, which must obviously occur in experiments in which the varying size and degree of distention of the gall-bladder are not controlled. The blood and lymph of the animal were then examined at fifteen minute intervals by the Van den Bergh [12] test for the presence of bilirubin. This test is said to be sensitive to bilirubin in a dilution of 1-1,500,000 in the blood serum and it can be applied in the fifteen minute intervals between the taking of the various specimens of blood and lymph. It was planned, moreover, to kill the animal as soon as a positive test for bilirubin was obtained, so that portions of the liver might be immediately fixed for microscopic study in which the ordinary hematoxylin and eosin stain as well as the Eppinger [1] and the Vance [13] specific stains for bile capillaries were used. The dog was the animal used in these experiments because the previous workers had used this animal, because of its desirably large size for the necessary operative work, and especially because the dog's serum is normally free from bilirubin.

RESULTS

Since the dog's kidney has such a low threshold for bilirubin that the pigment sometimes escapes in the urine under nearly normal conditions, it was necessary to determine whether removal of the kidneys alone would produce a bilirubinemia, as this procedure was to be a necessary part of later experiments. This point was, therefore, tested experimentally on four dogs. The following protocol is quite typical of all.

Dog Number 3, a male mongrel, weighing 9 kilograms, was anesthetized with morphine. Through a midline incision in the abdomen both kidneys were removed transperitoneally. The right carotid artery was then cannulated. The operation was begun at 10:18 a.m. and was finished at 10:53 a.m. Samples of blood taken at fifteen minute intervals until 5:30 p.m. remained consistently negative for bilirubin. The animal was then killed. The autopsy showed that there was no free blood within the abdominal cavity; the rest of the autopsy showed nothing abnormal. The results of this experiment on four dogs are summarized in Table I.

TABLE I.

Removal of kidneys not followed by a bilirubinemia.

Dog	Sex	Weight in Kg.	Length of Experiment	Bilirubin Test Every 15 Minutes
1	M	7	3 hours	Negative
2	M	5.5	4 hours 50 minutes	Negative
3	M	9	6 hours 37 minutes	Negative
8	F	4	3 hours 25 minutes	Negative

It may be seen from the above table that removal of both kidneys in each of four dogs did not produce a bilirubinemia at any time during observations continued as long as six and one-half hours.

After this, a series of experiments was performed to determine how soon bilirubin appeared in the serum of dogs from which both kidneys were removed, the common bile-duct cut between ligatures and the gall-bladder extirpated. The following experiment on one dog, which is reported in detail, is typical of those performed on eight others, the results of which are briefly presented in Table II.

Dog Number 7, male, mongrel, weighing 8 kilograms. Under morphine anesthesia the right carotid artery was cannulated at 12:15 p. m. A sample of blood taken at this time was negative for bilirubin. Through a midline abdominal incision both kidneys were removed at 12:32, following which the common bile-duct was cut between ligatures and the gall-bladder removed after ligation of the cystic duct. The ducts were tied at 12:55. Samples of blood taken at 1:45, 2:00 and 2:15 were negative for bilirubin. At 2:35 the blood gave a very faint indirect Van den Bergh test. Before a part of the liver was removed for microscopic study, a rapid inspection of the peritoneum showed that there had been no post-operative bleeding. The animal was killed with ether and the autopsy showed that the gall-ducts were distended. The liver was apparently normal in color and

TABLE II.

Earliest appearance of bilirubinemia after obstructing the common bile-duct in the absence of the kidneys and the gall-bladder.

Dog	Sex	Weight in Kg.	Time lapse between ligation of the common bile-duct and the first positive Van den Bergh test for bilirubin.		Remarks
			Direct Test	Indirect Test	
4	F	3	Negative	4 hrs.	Puppy
5	M	8	Negative	2 hrs.	
6	M	16	Negative	1 hr. 40 min.	
7	M	8	Negative	1 hr. 40 min.	
9	F	5	Negative	3 hrs. 10 min.	Slight bleeding in peritoneum
10	M	9	Negative	2 hrs. 25 min.	
11	F	10	Negative	1 hr. 20 min.	
12	F	10.5	Negative	2 hrs. 15 min.	
14	M	24	Negative	1 hr. 50 min.	

consistency; the remainder of the autopsy disclosed nothing abnormal. The results of the microscopic study will be discussed later.

The results of the experiments tabulated above show that bilirubin can first be detected in the blood approximately two hours after the ligation of the common duct. A significant finding in these experiments, which will be referred to below, is that the indirect Van den Bergh reaction alone was obtained; the direct reaction, which has hitherto been considered pathognomonic for obstructive jaundice, was consistently negative in these experiments.

A series of experiments was next performed in which the above procedures were duplicated and, in addition, the entire lymph of the thoracic duct was collected so that a comparison of the lymph and the blood for the presence of bile pigment could be made. The following is a protocol typical of the six experiments shown in Tables III and IV.

Dog Number 28, a large male mongrel weighing 16 kilograms, was anesthetized with one grain of morphine and ether. The lymph of the thoracic duct was obtained by ligating all the veins entering the junction of the left external jugular, subclavian and innominate veins and the insertion at 11:25 a. m. of a large cannula into the left external jugular vein. The lymph flowed easily and regularly at the rate of 25 c.c. an hour. The right femoral artery was then cannulated and a control specimen of blood was taken; this was found to contain no bilirubin. A midline incision was then made into the abdomen, both kidneys were removed, the gall-ducts were ligated and sectioned at 12:01 and the gall-bladder was removed. The lymph flow was good, being about 35 c.c. an hour. The ether was discontinued and the dog was given another half grain of morphine. The lymph was negative for bilirubin at 12:25; at 12:45 it was definitely and at 1:02 it was strongly positive. The blood serum remained negative for bile pigment until the dog was killed at 1:25; when sections of the liver were taken for microscopic study. At autopsy the operative field in the peritoneum was found to be free from blood, the bile-ducts were distinctly distended, the ligatures on the gall-ducts were securely placed, *the lymphatics at the hilum of the liver were dilated and distinctly yellow-green in color.* The thoracic duct was also of a deep yellow color.

TABLE III.

Bilirubin tests on blood and lymph after ligation of the common bile-duct and removal of the kidneys and gall-bladder.

Dog	Sex	Weight in kg.	Van den Bergh tests on blood	Time between occlusion of common bile-duct and appearance of bilirubin in lymph	
				Direct	Indirect
20	F	18	Negative	Negative	58 minutes
25	F	16.5	Negative	Negative	62 minutes
28	M	15	Negative	Negative	45 minutes

In the three experiments of Table IV the lymph flow was so scanty that the thoracic duct was ligated at its entrance into the left external jugular vein and the rest of the procedures of the experiments of Table III were carried out.

TABLE IV.

Bilirubin tests on the blood and lymph after ligation of the thoracic duct and the common bile-duct and removal of the kidneys and gall-bladder.

Dog	Sex	Weight in Kg.	Duration of Experiment	Van den Bergh tests on blood	Van den Bergh test on lymph from cisterna chyli at end of experiment.	
					Direct	Indirect
19	M	23	5 hrs.	Neg.	Neg.	Very strong
24	M	19	4 hrs.	Neg.	Neg.	Very strong
26	M	18	1 hr. 40 min.	Neg.	Neg.	Definitely positive

From the above two tables it is evident that, in acute obstructive jaundice in dogs from which the kidneys have been removed and the gall-bladder extirpated, a positive test for bilirubin is obtained in the lymph stream while the blood is still pigment-free. Moreover, in the three experiments in which the lymph stream of the thoracic duct was obstructed, the blood was pigment-free for periods up to five and one-half hours; at the end of which periods the animals were killed. In these three animals the cisternae chyli were greatly distended and distinctly yellow-green in color. It is to be noted again that in the lymph stream, just as in the previous experiments on the blood stream, only the indirect Van den Bergh reaction was obtained. This point will be discussed below.

The Microscopic Appearance of the Liver in the Earliest Stage of Obstructive Jaundice

The livers of the animals in which an obstruction to the passage of bile into the duodenum was produced, when fixed in either ten per cent formalin or in Zenker's fluid and then stained with the routine hematoxylin and eosin, show that many of the Kupffer cells are loaded with granules of green-yellow pigment. But the presence of these green granules is not constant for all of the sections. The bile capillaries were demonstrated by means of either the Eppinger or the Vance methods. It was found that satisfactory preparations could be obtained by fixing the liver tissue in equal parts of ten per cent formalin and Zenker's fluid without the acetic acid, embedding in celloidin, and staining according to Eppinger's method. This procedure results in a saving of several weeks' time in the preparation of the section. In all of the sections it is interesting to find that the normally thin, branching, intercellular bile canaliculi are quite thick and tortuous. But after diligent and careful search no bile capillaries were found that seemed to be ruptured; the processes of the canaliculi extending towards the blood capillaries always ended in distinct bulbs and, moreover, very rarely extended as far as the tissue spaces surrounding the liver cell columns. In animals in which the common duct has been tied for three and four days, the bile canaliculi are also apparently not ruptured. Certainly there is nothing which at all approximates the pictures which Eppinger[1] and others have obtained in long-standing mechanical jaundices. The condition of

the bile capillaries in these experiments are in accord with the findings of Jagič [3] and Ogata.[4]

The application of the Van den Bergh test to the blood of patients suffering with jaundice has called forth, during the past few years, a long series of papers dealing with the different types of reaction given by the sera of patients ill with jaundices of different etiology (Lephene,[14] Feigl and Querner,[15] McNee [16]). To date the data in hand indicate that the direct reaction—a red-violet color resulting from the addition of Ehrlich's diazo reagent to the serum—is given only by jaundices of out spoken obstructive origin. The indirect reaction, that is, a pink color resulting from the addition of the diazo reagent to alcoholized protein-free serum, is given by all types of jaundice regardless of the etiology. Then, too, an attempt has been made to link up the urinary findings with those of the blood. It has been known for a long time that patients with an obstructive jaundice, that is, those whose serum would give both the direct and indirect reactions, have bilirubin in the urine; while, on the other hand, patients suffering with hemolytic jaundices, the type that gives the indirect reaction alone, have urobilin in the urine but no bilirubin. In view of the clinical interpretations of the Van den Bergh test, it was indeed surprising to find that in producing an obstructive jaundice, the reaction, in the early stages at least, was that of a "hematogenous" icterus, that is, the indirect one. In view of these facts it was decided to section the ductus choledochus aseptically in the dog and to watch the blood serum for the appearance of the direct Van den Bergh reaction and to observe the urine at the same time for the presence of either bilirubin or urobilin. It is intended to make a complete report of these experiments at a later date. It was felt desirable, however, to mention the results of this work in so far as it applied to the length of duration of the indirect and the time of appearance of the direct reaction after section of the bile-duct.

In these experiments the ductus choledochus was sectioned under conditions of surgical asepsis. The blood was then examined every day for its reaction to the Van den Bergh test, and the urine, obtained by catheterization, was examined daily for the presence of urobilin and bilirubin. When the animals were killed, the livers were prepared for staining with hematoxylin and eosin and the Eppinger stain for bile capillaries. The results so far indicate that the indirect reaction, although quantitatively progressively stronger each day, lasts for two to four days after the section of the duct. Then the direct reaction appears. It was found that removal of the gall-bladder at the time that the common duct is sectioned hastens the appearance of the direct reaction by at least one day. These experiments would not have been mentioned but for the fact that they show that the indirect Van den Bergh precedes the direct reaction by several days in early obstructive jaundice, a fact which, as far as I have been able to find, has never been observed and deserves more careful investigation. The relationship of the appearance of the direct test to the question of the integrity of the bile canaliculi has not been fully worked out as yet; it is intended to report later on this question as well as on the pigments found in the urine.

Discussion

The results of the experiments reported in this paper must cause, it is felt, a revision of some of the ideas held at present on the processes involved in early obstructive jaundice. In the first place, it has been shown that, contrary to the prevailing idea, but in accord with the work of Jagič and Ogata, the fine intercellular bile capillaries are not ruptured before there is an appreciable bilirubinemia consequent to the occlusion of the common duct. In the second place, the blood serum of dogs in the first day or two after the blocking of the ductus choledochus gives the indirect Van den Bergh reaction, and in so far renders doubtful the interpretation of the indirect test when actual tissue icterus is not present. On the other hand, after the sclerae are definitely icteric the direct test is always given.

It is felt, moreover, that the ready applicability of the Van den Bergh test to the serum and the lymph, coupled with the removal of the kidneys and the gall-bladder have made it possible to decide much more definitely than has been done in the past that the removal of bile pigment from the liver in the earliest stages of mechanical jaundice is entirely by the lymph stream and not by the blood stream.

I am indebted to Dr. Arnold R. Rich for the interest and valuable suggestions which he has contributed to the carrying out of this investigation.

Summary

1. During the first hours after the occlusion of the common bile-duct the bile pigments are carried from the liver by the lymph stream and by it are emptied into the general circulation.

2. In none of the above experiments were ruptured bile canaliculi found during the earliest stages of mechanical jaundice.

3. During at least the first two days after the occlusion of the common bile-duct the blood serum gives only the indirect and not the direct reaction which is claimed to be specific for obstructive jaundice.

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ERRATA

In Dr. Dandy's article in the August Bulletin, paragraph 4 on page 245 should read "The function of cerebrospinal fluid is principally a mechanical one, and in this paper I shall discuss its adaptability to acute and chronic disturbances within the cranio-vertebral chamber." In the second paragraph on page 247, instead of "symmetrical" read "asymmetrical." On page 249, line 21, instead of "centre" read "cortex." Under Figure 1, page 250 should appear the legend:

Fig. 1.—Diagram illustrating the method by which the cerebrospinal spaces compensate for any loss of brain tissue. (A) A defect in or near the cortex is compensated by dilatation of the superimposed subarachnoid spaces (A^1), Cf. Fig. 5. (B) A defect midway between the ventricle and the surface of the brain is compensated by dilatation of the subarachnoid space over the defect and ventricular dilatation under the defect (B^1). (C) A defect deeply seated in the brain is compensated by dilatation of the ventricle (C^1). (D) Atrophy of the whole thickness of the brain is compensated by a great diverticulum of the ventricle. In some cases, this may assume tremendous proportions.

NOTES ON NEW BOOKS

Venereal Diseases:—Their Clinical Aspect and Treatment. J. E. R. McDonagh, F.R.C.S. $20.00. (*St. Louis, C. V. Mosby Company, 1920.*)

Mr. McDonagh is the iconoclast of syphilology. He is the sponsor for many exceedingly original ideas, chief among which are "that the spirochæta pallida is only the adult male of a coccidial protozoon; that the complement fixation test is merely a physical reaction dependent upon the increased number and size of the protein colloidal particles in the serum; and that salvarsan only destroys the parasite indirectly, by increasing the oxidising action of the host's protective substance."

This volume does not enter into the detailed proofs of his views and is composed of clinical descriptions of syphilis, gonorrhea, chancroid, and other rarer venereal diseases. The most interesting parts of the book are of course those dealing with syphilis. It may be said that, from the purely clinical standpoint, McDonagh's work is excellent. As a matter of fact, although no attempt is made to give exhaustive descriptions of the rarer manifestations of syphilis, the disease as a whole is well covered.

The chapters on the biology of syphilis of the nervous system, its clinical aspect, and on syphilis in women, are especially good, and show a firm grasp of the point of view of syphilis as a disease entity. Too little stress is laid on aortic and cardiac syphilis, which are quite as important to the patient as neurosyphilis. The illustrations, including 68 colored plates, are also good, though unfortunately their presence renders the price of the book almost prohibitive.

The chief drawback to this work is the fact that the author intersperses his accurate clinical observations with statements of his own startling views, most of which have never been confirmed by the work of others. He claims to be able to prognosticate the future course of syphilis from the clinical appearance of the chancre, stating that one type of chancre is usually followed by recurrences, another by nervous syphilis, another by vascular lesions, etc. The different ways in which his *leucocytozoon syphilidis* may develop in a chancre, thus affecting not only its appearance, but also the later course of the disease, are described, but for these and other similar observations not the slightest iota of proof is adduced. He gives detailed outlines for the treatment of syphilis (and also of gonorrhea) with the usual drugs, plus colloidal solutions of various metals invented by himself. These are intramine (colloidal sulphur), ferrivine (colloidal iron), colloidal iodine, colloidal mercury, pallamine (colloidal palladium), trimine (a colloidal mixture of manganese, iron, and zinc), and others. He does not give the composition of these drugs (except for intramine, of which the formula is stated), or the results of toxicity tests, or of their experimental use. He does, however, state briefly his theory of their use, which is too lengthy to enter into a review.

On the whole, from the clinical standpoint, the book is excellent, and the numerous interpolations of the author's unique and as yet unsubstantiated views render it of great interest to the special worker in the field of syphilogy. It is, however, questionable if this method of presenting the subject is suitable for the use of students.

J. E. M.

Diseases of Infancy and Childhood. By Louis Fischer. Ninth Edition. $12.00. (*Philadelphia, F. A. Lewis Co., 1922.*)

Most of the recent text-books on Pediatrics fall into one of two groups; they are either sketchy and incomplete, or stuffy, overlong and tiresome. Fischer's "Diseases of Infancy and Childhood" strikes an admirable medium. While not a system of pediatrics, it is, nevertheless, rēmarkably complete and amply full enough in its treatment of subjects to act as a reference book for general practitioners and students. It is up-to-date, but conservative; it mentions all the important features of each condition briefly and clearly, at the same time avoiding fruitless theorizing.

The illustrations are good; some of the colored plates are very good, particularly those illustrating the eruptive fevers and the various cutaneous reactions. On the whole, this book, although not planned particularly for the specialist, should prove a valuable addition to his library. The general practitioner will find it an extremely useful and safe guide.

B. T.